Université de Montréal

Allélotypage des gènes de réparation de l'ADN dans les cancers non-héréditaires

par

Sébastien Guiral

Programme de biologie moléculaire

Faculté des études supérieures

Mémoire présenté à la Faculté des études supérieures
en vue de l'obtention du grade de
Maître ès en sciences (M.Sc.)
en biologie moléculaire

Septembre, 1998

Page d'identification du jury

Université de Montréal

Faculté des études supérieures

Ce mémoire intitulé :

Allélotypage des gènes de réparation de l'ADN dans les cancers non-héréditaires

présenté par :

Sébastien Guiral

Edward Bradley
Daniel Sinnett
marc Basik

mémoire accepté le 05.04.99

SOMMAIRE

Les cancers du sein, du poumon et du côlon sont très fréquents dans le monde occidental. Au Canada, ces trois néoplasies représentent près de 45% des nouveaux cas de cancer. Les tumeurs sporadiques, qu'elles apparaissent spontanément ou qu'elles soient induites par l'exposition à des facteurs externes, sont le résultat d'un processus multi-étape. Elles se développent suite à l'accumulation d'altérations génétiques qui modifient, entre autres, l'expression d'oncogènes et de gènes suppresseurs de tumeurs. Récemment, des mutations au niveau d'un groupe de gènes codant pour des composantes du système de réparation de bases mésappariées (**SRM**) ont été associées à une prédisposition génétique à différentes formes de cancers. L'altération de ces gènes mène à une augmentation du taux de mutations spontanées dans les cellules cancéreuses donnant ainsi naissance à un phénotype mutateur (instabilité génétique). Le but de ce projet était de déterminer l'importance des gènes du système de réparation de bases mésappariées dans le développement des cancers sporadiques.

Pour atteindre ce but, nous avons déterminé les profils alléliques (allélotypage) des régions associées à 6 gènes du système de réparation de bases mésappariées, (*hMLH1, hMSH2, hMSH3, hMSH6, hPMS1* et *hPMS2*) chez des patients atteints de la forme sporadique des cancers du sein, du poumon et du côlon. L'allélotypage, effectué par PCR à l'aide de marqueurs microsatellites, nous a permis d'identifier simultanément les tumeurs exprimant un phénotype mutateur et/ou celles montrant des pertes d'hétérozygotie.

L'expression d'un phénotype mutateur, révélée par une instabilité des microsatellites, a été observée chez aucun cancer du sein (0/22) et chez seulement 3% (1/31) des tumeurs du poumon. La présence de délétions hémizygotes a été observée dans 46% et 23% des cas de cancer du sein pour les loci d'*hMLH1* et d'*hMSH3*, alors que 55% et 42% des cas de cancer du poumon autres qu'à petites cellules montraient des pertes

alléliques aux mêmes loci. L'analyse de plusieurs marqueurs microsatellites a permis d'identifier des régions communes de délétion qui s'étendent sur un intervalle génétique d'environ 1 cM. À ce jour, les seuls gènes candidats connus à l'intérieur de ces régions sont *hMLH1* et *hMSH3*. L'analyse mutationelle par *SSCP* (*Single-Strand Conformational Polymorphism*) des cas ayant des pertes alléliques au niveau de ces loci n'a pas révélé de mutation inactivante au niveau de l'allèle résiduel non-délété. Toutefois, nous avons identifié deux nouveaux polymorphismes à l'intérieur de la séquence codante du gène *hMSH3*.

Chez les cancers colorectaux, nous avons décelé l'expression d'un phénotype mutateur dans 11% (4/35) des cas étudiés. Aucune corrélation n'a été observée entre la présence de ce phénotype et les délétions hémizygotes observées dans 17% des cas pour *hMLH1*, 15% pour *hMSH2/hMSH6* et 18% pour *hMSH3*. Toutefois, l'analyse du statut de méthylation du promoteur du gène *hMLH1* a révélé que cette région était méthylée dans 75% des cas exprimant un phénotype mutateur.

À la lumière de ces résultats, nous suggérons que les gènes *hMLH1*, *hMSH3* et *hMSH2/hMSH6* sont impliqués dans la tumorigenèse du sein, du poumon et du côlon par un effet de dosage affectant des processus cellulaires distincts de la réparation d'erreurs commises lors de la réplication. En effet, ces gènes de réparation sont impliqués dans plusieurs autres processus cellulaires vitaux. Nous suggèrons aussi que la méthylation du promoteur constituerait un mécanisme important d'inactivation de *hMLH1* dans les cas 'instables' de cancer sporadique du côlon. L'élucidation du rôle des gènes du système de réparation de bases mésappariées dans la tumorigenèse permettra d'améliorer notre compréhension de la susceptibilité génétique au cancer sporadique.

TABLE DES MATIÈRES

TABLE DES MATIÈRES (suite)

LISTE DES TABLEAUX

LISTE DES FIGURES

LISTE DES SIGLES ET ABRÉVIATIONS

ADN : Acides déoxyribonucléiques

SRM : Système de réparation de bases mésappariées

PCR : Polymerase chain reaction

hMLH1 : Human *MutL* homolog 1

hMSH2 : Human *MutS* homolog 2

hMSH3 : Human *MutS* homolog 3

hMSH6 : Human *MutS* homolog 6

hPMS1 : Human post-meiotic segregation 1

hPMS2 : Human post-meiotic segregation 2

GTBP : G/T binding protein

SSCP : Single-strand conformational polymorphism

PDH : perte d'hétérozygotie

BER : Base excision repair

NER : Nucleotide excision repair

TCR : Transcription-coupled nucleotide excision repair

HNPCC : Hereditary non-polyposis colon cancer

CPAPC : Cancer du poumon autres qu'à petites cellules

CPPC : Cancer du poumon à petites cellules

SCLC : Small cell lung cancer

RER : Replication error

INTRODUCTION

En Amérique du Nord, les cancers du poumon, du côlon/rectum et de la prostate sont les trois cancers les plus répandus chez l'homme ; chez la femme, ce sont les cancers du poumon, du côlon/rectum et du sein qui arrivent en tête (Statistique de l'Institut national du Cancer du Canada, 1998). En 1998, on estime à 62,700 le nombre de décès attribuables à cette maladie dont le principal site est le poumon (Statistique de l'Institut national du Cancer du Canada, 1998). Au cours de la vie, 1 femme sur 9 sera atteinte du cancer du sein tandis qu'une sur 18 sera atteinte d'un cancer du côlon/rectum et 1 sur 21 présentera un cancer du poumon. Chez les hommes, 1 sur 8 développera un cancer de la prostate et 1 sur 11 sera atteint du cancer du poumon.

LE CANCER EST UN PROCESSUS MULTI-ÉTAPE

En 1976, Nowell a définit la croissance d'une tumeur comme une évolution clonale propulsée par la force des mutations. Depuis, il a été établit que l'apparition d'une tumeur est le résultat d'un processus à plusieurs étapes (Vogelstein et Kinzler, 1993) à travers lesquelles des changements génétiques (mutations) progressifs amènent une cellule normale à perdre les mécanismes de contrôle de sa prolifération. L'incidence de cancer qui augmente avec l'âge illustre le temps nécessaire pour accumuler le nombre requis de mutations pour qu'une cellule normale devienne maligne (Vogelstein et Kinzler, 1993). La tumorigenèse du côlon est le paradigme de ce processus de progression à plusieurs étapes (Figure 1, pp. 3). Les cancers colorectaux se développent suite à l'apparation de mutations successives au niveau des gènes *APC, kRas* et *p53* (Cho et Vogelstein, 1991). Ce modèle génétique fait ressortir trois points importants de la tumorigenèse : (1) les tumeurs

progressent grâce à l'activation d'oncogènes et à l'inactivation de gènes suppresseurs de tumeurs, (2) des mutations dans plusieurs gènes sont requises pour obtenir une transformation maligne des cellules, (3) même si les propriétés biologiques d'une tumeur dépendent de l'accumulation de changements génétiques plutôt que leur ordre d'apparition, une première mutation au niveau d'un gène qualifié de '*Gatekeeper*' semble être requise pour l'initiation d'un cancer. Plus particulièrement, chaque cancer semble avoir son gène '*Gatekeeper*' : côlon = *APC*, rétinoblastome = *RB*, tumeur de Wilms = *WT1*, etc (Cho et Vogelstein, 1991 ; Kinzler et Vogelstein, 1997).

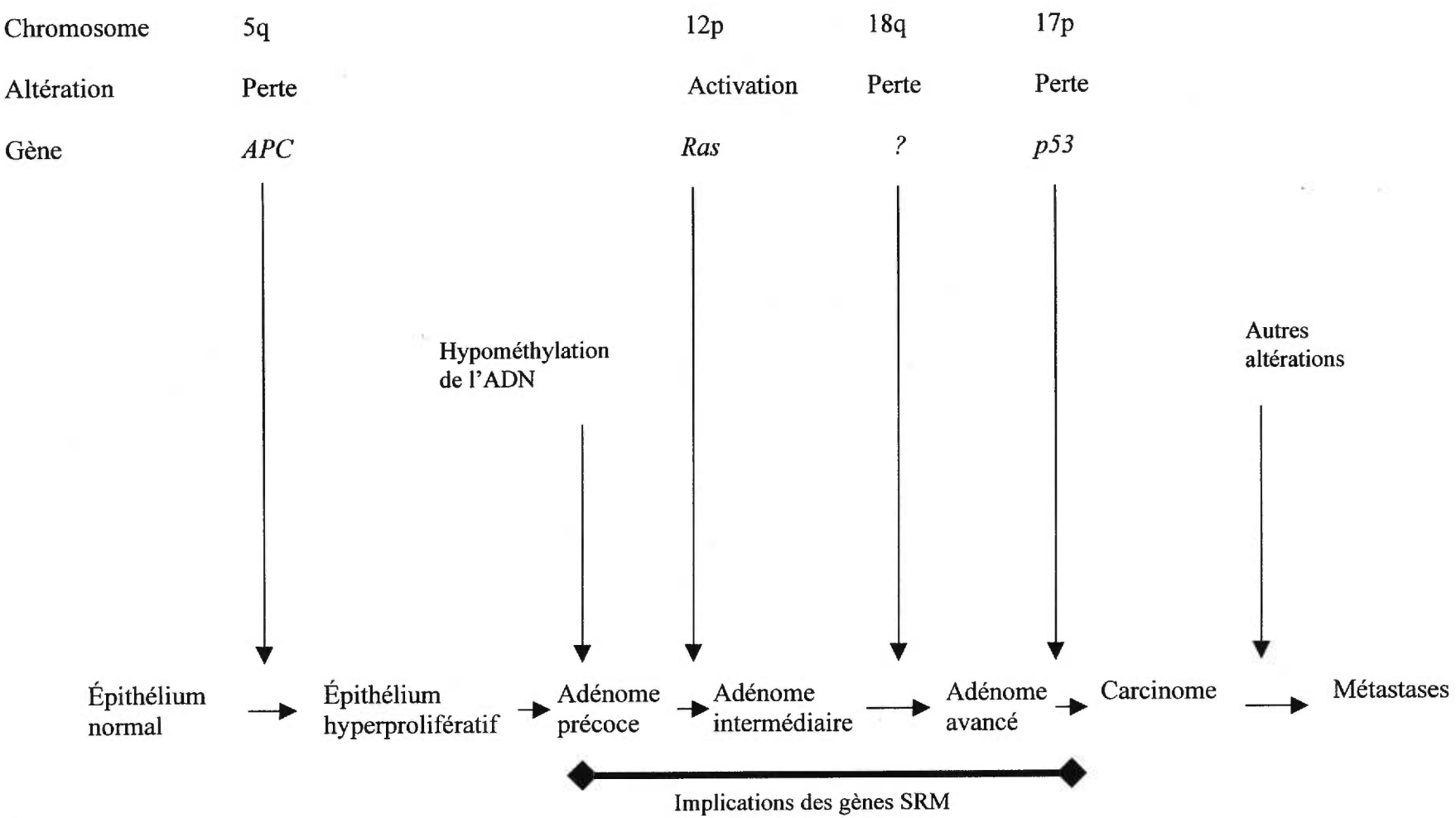
En résumé, un nombre 'critique' d'événements génétiques doivent se produire pour qu'un cancer progresse (Fong *et al.*, 1995). La nature de ces mutations est diverse incluant des amplifications, des mutations ponctuelles, des réarrangements ainsi que des délétions (Cho et Vogelstein, 1991). L'altération de gènes suppresseurs est généralement un des événements les plus précoce dans le développement d'une tumeur (Yokota et Sugimura, 1993). L'étude de ce type de gène permet donc d'améliorer notre compréhension des causes du cancer.

GÈNES SUPPRESSEURS DE TUMEURS

ORIGINE

La recherche sur les causes et le processus de carcinogenèse a pris son essor au cours des trois dernières décennies grâce aux progrès dans le domaine de la génétique moléculaire. Depuis le milieu des années 1980, une attention particulière a été portée à un groupe de gènes connus comme oncogènes récessifs, antioncogènes ou gènes suppresseurs de

Figure 1. Modèle génétique de la tumorigenèse du côlon



Modifié de Vogelstein et Kinzler, 1993.

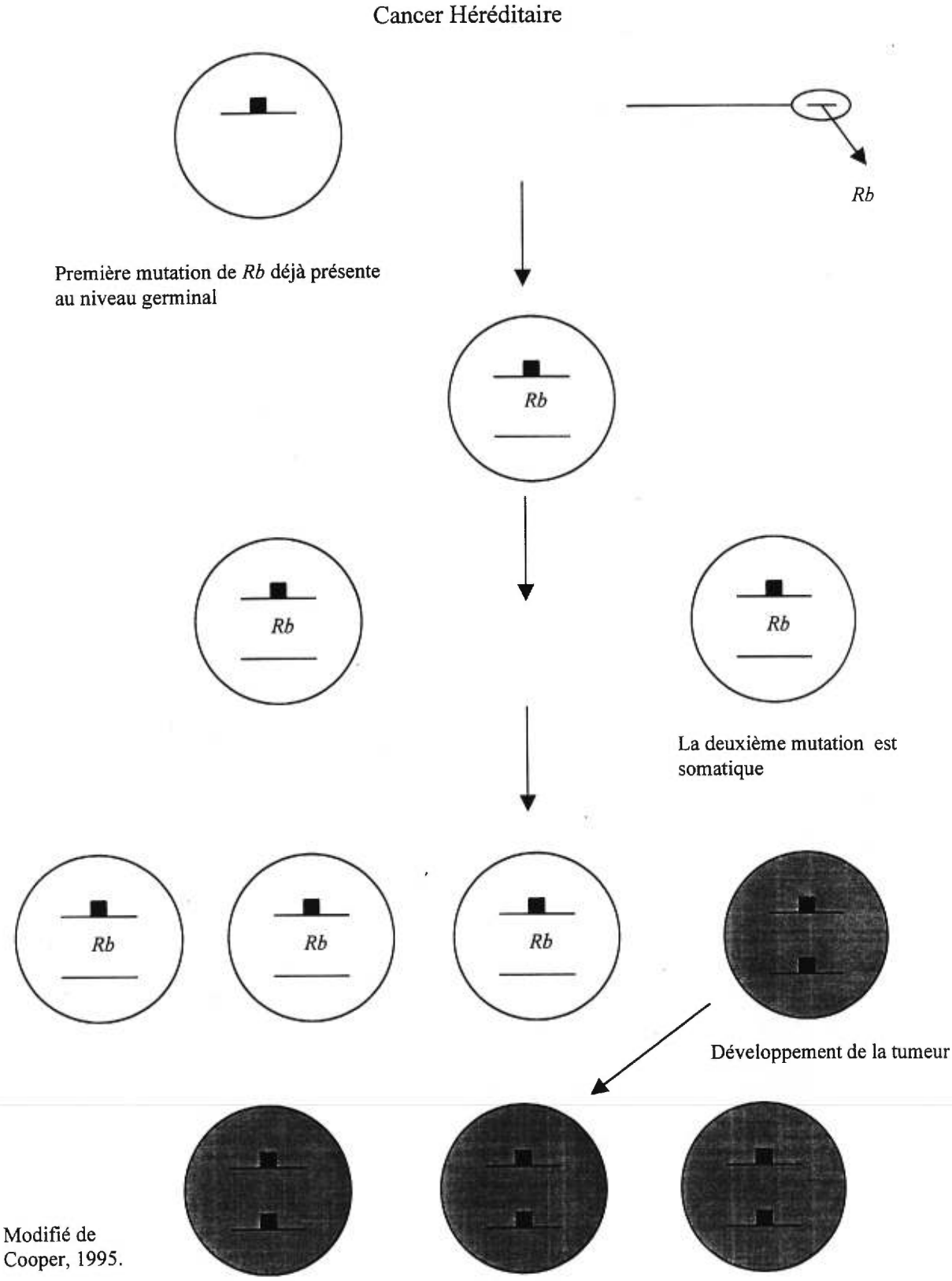
tumeurs. Comme les noms l'indiquent, ces gènes codent pour des composantes des mécanismes impliqués dans le contrôle de la prolifération cellulaire (Knudson, 1993). Le concept de gènes suppresseurs de tumeurs remonte à 1929 lorsque Boveri a suggéré qu'une cellule pouvait devenir maligne soit par la prédominance de chromosomes promoteurs de division cellulaire ou bien par l'élimination de chromosomes inhibiteurs de ce processus (Boveri, 1929). Par la suite, les études épidémiologiques de Knudson (1971) sur la tumeur pédiatrique de rétinoblastome ont amené l'idée de gènes suppresseurs de tumeurs. Dans son modèle, Knudson (1971) proposait que dans les cas héréditaires, une prédisposition à développer un rétinoblastome venait de la présence d'une mutation germinale au niveau d'un des allèles du gène suppresseur de tumeurs (cellule prédisposée) et que l'inactivation de l'autre allèle lors d'un événement mutationnel somatique menait au déclenchement du processus tumoral (Figure 2, pp. 5). Pour les cas sporadiques, ce modèle suggère que les deux allèles sont inactivés par deux événements somatiques (Figure 3, pp. 6).

Ensuite, divers travaux de génétique moléculaire ont permis la localisation et l'identification du gène *RB1* responsable du rétinoblastome (Cavenee *et al.*, 1983, 1985) qui est ainsi devenu le paradigme des gènes suppresseurs de tumeurs (revue dans Kok *et al.*, 1997).

IDENTIFICATION DES GÈNES SUPPRESSEURS DE TUMEURS

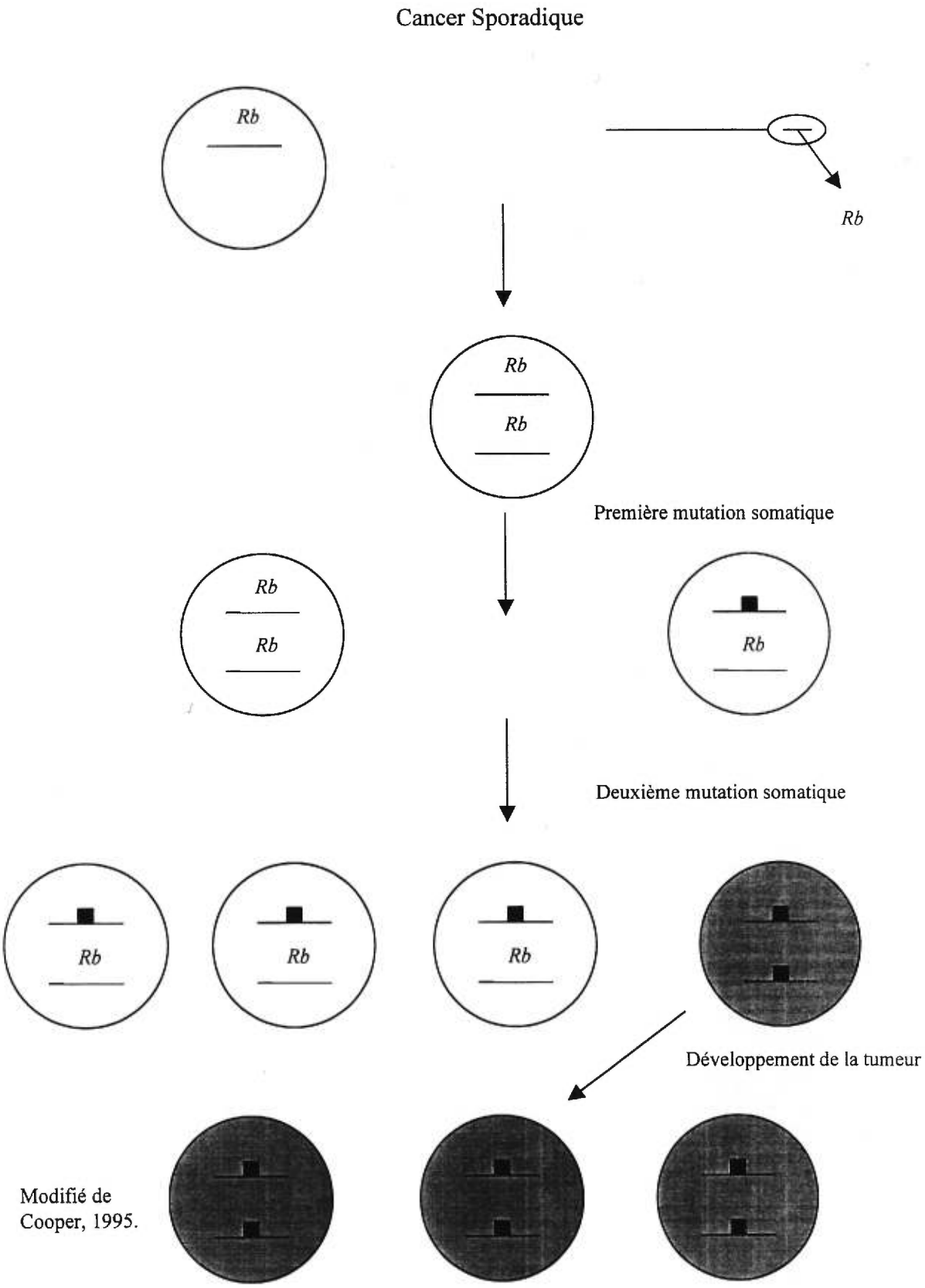
Étant donné leur importance dans la tumorigenèse, l'identification de gènes suppresseurs de tumeurs est essentielle à la compréhension de ce processus. Plusieurs méthodes sont utilisées pour atteindre ce but : analyse cytogénétique, détection de pertes alléliques et études de fusions cellulaires et de transfections (revue dans Kok *et al.*, 1997). À cet égard,

Figure 2. Modèle de Knudson pour l'inactivation d'un gène suppresseur de tumeurs pour un cancer héréditaire



Modifié de Cooper, 1995.

Figure 3. Modèle de Knudson pour l'inactivation d'un gène suppresseur de tumeurs pour un cancer sporadique

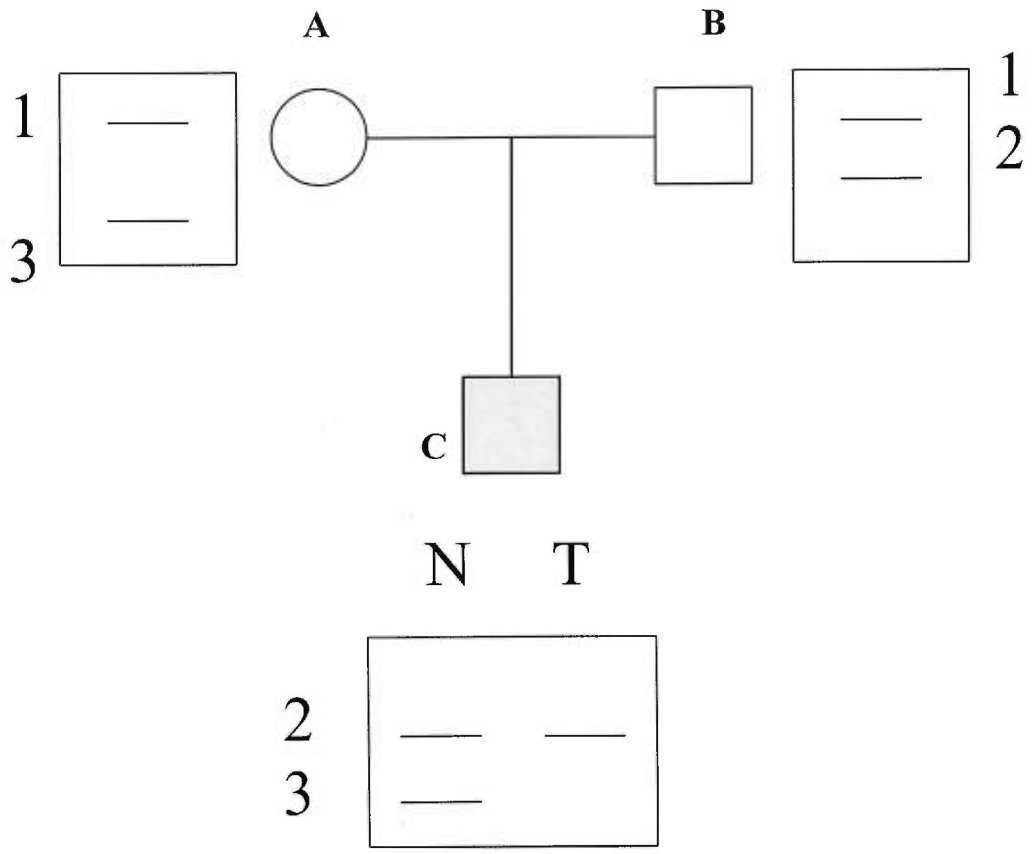


l'approche la plus populaire est certe la recherche de pertes d'hétérozygotie (PDH) dans les cellules tumorales. Le processus d'inactivation en deux étapes des gènes suppresseurs de tumeur, implique souvent une délétion comme deuxième événement. Cependant, il est aussi possible qu'une PDH soit acquise de façon alléatoire et n'est aucune association avec le processus tumoral (Vogelstein *et al.*, 1989 ; Chen *et al.*, 1992). Un niveau de base de PDH est présent dans les cellules cancéreuses (Vogelstein *et al.*, 1989) et par ce fait seulement les PDH présentent à un niveau excédant grandement le niveau de base sont considérées comme marquant la présence d'un gène suppresseur de tumeur (Vogelstein *et al.*, 1989 ; Chen *et al.*, 1992 ; Kok *et al.*, 1997). La présence de PDH est décelée par l'entremise d'une analyse de marqueurs polymorphiques, plus particulièrement des marqueurs microsatellites. Les séquences microsatellites sont caractérisées par des petites répétitions en tandem de type $(GT)_n$ distribuées aléatoirement à travers le génome humain (Weber *et al.*, 1989). La nature polymorphique de ces loci est due à la variation du nombre de répétition en tandem qui est variable dans la population. L'analyse de ces marqueurs permet d'obtenir un profil allélique spécifique à chaque individu et pour chaque locus. La comparaison du profil allélique généré à partir des cellules tumorales versus les cellules normales permet de visualiser la présence de PDH (Figure 4, pp. 8). La grande densité de marqueurs microsatellites présentement disponible permet d'augmenter l'informativité des analyses de PDH, en plus de permettre la détection de petites régions délétées dans les tumeurs (Kok *et al.*, 1997). Ces analyses ont permis d'identifier plusieurs gènes répresseurs (Tableau I, pp. 9), ainsi que de nombreux loci candidats (Tableau II, pp. 10).

MÉCANISMES D'INACTIVATION DES GÈNES SUPPRESSEURS DE TUMEURS

L'inactivation des gènes suppresseurs de tumeurs peut se faire via divers mécanismes incluant des mutations ponctuelles, des réarrangements ou bien par la délétion d'un allèle. D'ailleurs, comme mentionné ci-dessus, la présence de pertes alléliques est un bon indicateur de la présence d'un gène répresseur. Récemment, il a été démontré que les gènes suppresseurs peuvent être inactivés par une modification épigénétique du promoteur.

Figure 4. Exemple de perte d'hétérozygotie (PDH)



A. Parent démontrant le profil allélique 1-3 pour un marqueur microsatellite.

B. Parent démontrant le profil allélique 2-3 pour le même marqueur microsatellite qu'en A.

C. Patient atteint d'une tumeur démontrant le profil allélique 2-3 au niveau de l'échantillon normal (N) et une perte d'hétérozygotie (perte de l'allèle 3) au niveau de l'échantillon tumoral (T) pour le même marqueur microsatellite qu'en A.

Tableau I. Exemples de gènes suppresseurs de tumeurs connus

Gène	Localisation Chromosomique	Cancer Héréditaire	Cancer Sporadique
RB	13p14	rétinoblastome	rétinoblastome, sarcome, cancer de la vessie, du sein, du poumon, de l'oesophage
p53	17p13	syndrome de Li-Fraumeni	cancer de la vessie, du sein, du côlon de l'oesophage, du foie, du poumon, des ovaires, tumeur du cerveau, sarcome, lymphome, leucémie
APC	5q21	*FAP**	cancer colorectaux, de l'estomac, du pancréas
MCC	5q21		cancer colorectaux
WT1	11p13	tumeur de Wilms	tumeur de Wilms
NF1	17q11	neurofibromatose de type 1	astrocytome et cancer du côlon
NF2	22q12	neurofibromatose de type 2	shwannome et méningiome
VHL	3p25	syndrome de von Hippel-Lindau	cancer du rein
MTS1	9p21	mélanome	mélanome, tumeur du cerveau, leucémie, sarcome, cancer de la vessie du sein, du rein, du poumon, des ovaires
BRCA1	17q21	cancer du sein et des ovaires	
BRCA2	13q12-q13	cancer du sein	
RET	10q11	néoplasie endocrine multiple 2	*MTC**, phaeochromocytome
CDK4	12q13	mélanome héréditaire 2	mélanome

* *FAP* : familial adenomatous polyposis, *MTC* : medullary thyroid cancer.

Tableau II. Loci candidats pour la présence de gènes suppresseurs de tumeurs dans les cancers

Bras chromosomique (1)	Cancer (2)
1p	sein, foie, estomac, côlon, mélanome, neuroblastome
1q	sein, poumon
3p	sein, poumon, rein, vessie, testicules, ovaires, cancer du cou et de la tête
4q	poumon
5q	sein, poumon, côlon
6q	sein, poumon, rein, ovaires, mélanome
7q	sein, ovaires, prostate, côlon, estomac
8p	sein, poumon, foie, côlon, prostate, ovaires
9p	poumon
10q	poumon
11p	sein, poumon, foie, vessie, ovaires, testicules
11q	sein, ovaires, côlon, cervix
12p	leucémie lymphoblastique aïgue
13q	sein, poumon, estomac, rein, ovaires
16q	sein, foie, ovaires, prostate
17p	sein, poumon, estomac, côlon, rein, vessie, ovaires
17q	côlon, ovaires, sein
18q	sein, estomac, rein, côlon, prostate, ovaires
22q	sein, foie, côlon, ovaires

1. Position de gènes candidats déduite par la présence d'altérations (ex. PDH) au niveau de ces régions chromosomiques.
2. Tumeurs démontrant des altérations au bras chromosomique indiqué.

Contrairement aux mécanismes mutationnels classiques, la séquence nucléotidique n'est pas altérée dans ce cas. L'hyperméthylation d'îlots CpG présents dans la région promotrice du gène mène à l'inactivation de ce dernier en réprimant la transcription (Ahuja *et al.*, 1997). Certains cas de rétinoblastome montrent une hyperméthylation du gène *RB* (Sakai *et al.*, 1991) et une association entre l'hyperméthylation et l'arrêt de transcription du gène *p16* a été trouvée chez des gliomes, des cas de cancer du poumon à petites cellules ainsi que chez des cas de cancer de la vessie (Gonzales-Zulueta *et al.*, 1995 ; Herman *et al.*, 1995 ; Merlo *et al.*, 1995). L'hyperméthylation du gène *VHL* a été observée pour plusieurs cas de cancer du rein (Herman *et al.*, 1994) et aussi chez des individus atteints du syndrome de von Hippel-Lindau (Prowse *et al.*, 1997). Dans ce dernier cas, l'inactivation du premier allèle vient d'une mutation germinale classique et l'événement somatique qui mène à l'inactivation du gène est la méthylation du deuxième allèle (Prowse *et al.*, 1997).

En d'autres mots, plusieurs formes d'altérations, génétiques et épigénétiques, peuvent entrer en jeu lors de l'inactivation d'un gène suppresseur.

TYPES DE RÉPARATION DE L'ADN

La réparation de dommages à l'ADN est un processus vital pour éliminer les mutations pouvant mener à l'initiation d'un cancer. Les cellules eukaryotes possèdent plusieurs mécanismes de réparation de l'ADN afin de maintenir l'intégrité du matériel génétique. La réparation par excision constitue une des stratégies employées pour faire la correction de dommages multiples à l'ADN (Friedberg, 1996). Cette réparation par excision est divisée en deux groupes d'après leur mécanisme d'action et la différence des produits qu'ils éliminent : (1) *base excision repair* (BER) (2) *nucleotide excision repair* (NER)

(Friedberg, 1996). Durant ce processus, les bases ou les nucléotides qui sont chimiquement modifiés, mésappariés, ou inappropriés sont retirés de l'ADN et remplacés par des bases de compositions et de séquences normales (Friedberg, 1996). De façon générale, le système de BER élimine les dommages à l'ADN qui peuvent se produire dans la cellule par l'entremise d'événements hydrolytiques comme la déamination ou la perte de bases, l'attaque de celles-ci par des radicaux libres d'oxygène et la méthylation d'azotes au niveau des anneaux des bases par des agents endogènes (Wood, 1996). De son côté, le système de NER répare notamment les dimères de pyrimidines causés par la radiation ultra-violette (Friedberg *et al.,* 1994) et ce à l'intérieur d'oligonucléotides allant de 25-32 nucléotides (Wood, 1996). Il est aussi reconnu que le système de NER peut être divisé en deux voies cinétiquement différentes : (1) un processus global de réparation qui retire les lésions présentes dans l'ensemble du génome et (2) un processus de réparation spécifique au brin transcrit de gènes actifs connu sous le nom de *Transcription-Coupled Nucleotide Excision Repair* (TCR) (Mueller et Smerdon, 1996).

Un troisième système de réparation impliqué dans la correction des erreurs survenues lors de la réplication semble jouer un rôle majeur dans l'étiologie de certains cancers. Le système de réparation de mésappariements de bases de l'ADN (**SRM**) est impliqué dans la reconnaissance et la correction de nucléotides qui sont incorrectement pairés (Fishel et Kolodner, 1995). Ces erreurs de pairage apparaissent dans le génome lors d'incorporations fautives de la polymérase au cours de la réplication ou par des dommages physiques aux nucléotides existants ou bien lors de la formation d'intermédiaires au cours de la recombinaison génétique (Fishel et Kolodner, 1995). Le SRM corrige ces erreurs et prévient ainsi la génération de mutations. Il est maintenant reconnu qu'un processus de correction de ces erreurs a été conservé à travers l'évolution, car on retrouve chez les

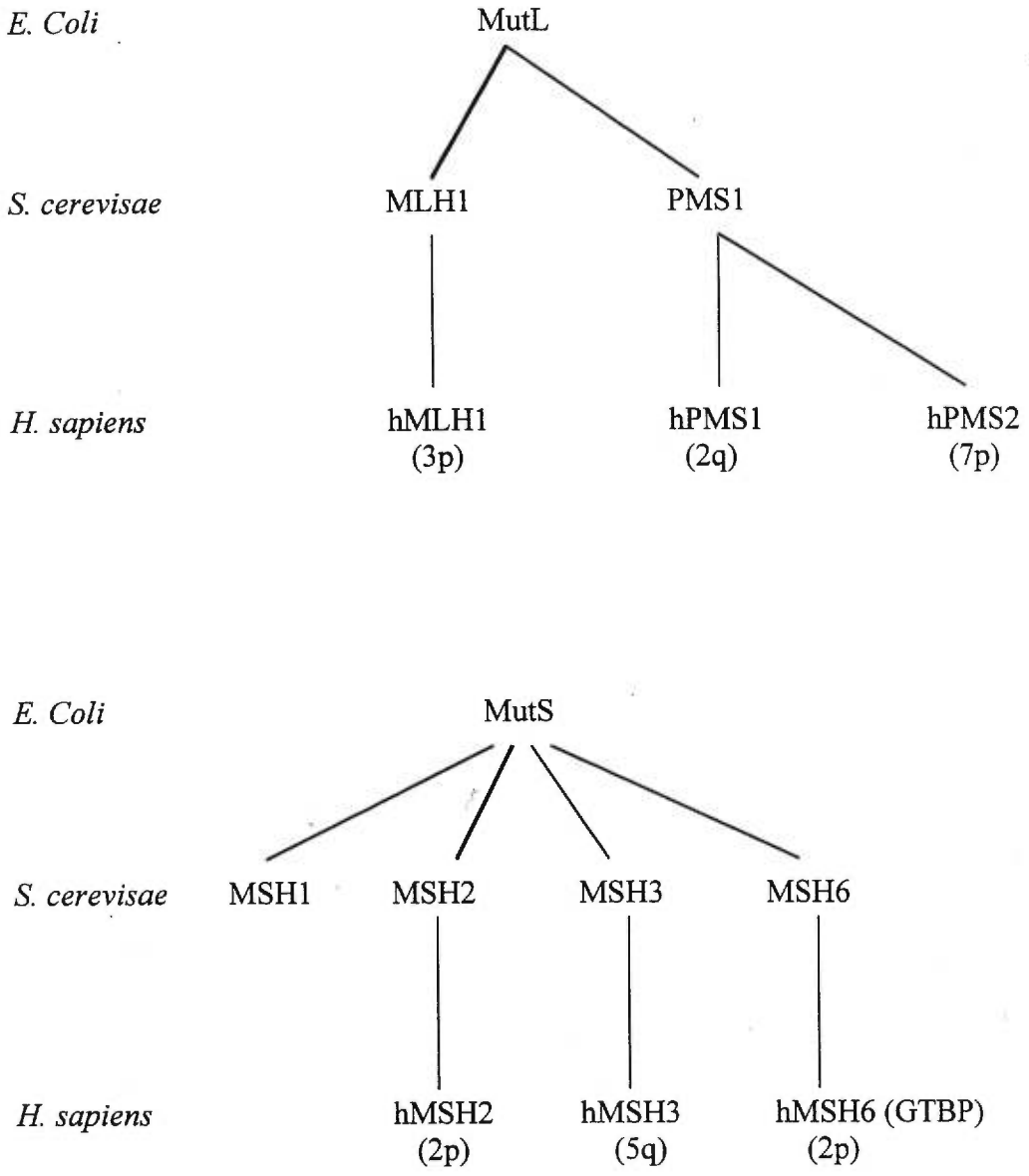
eukaryotes un système pour échapper aux mutations qui est semblable mais plus complexe que celui trouvé chez la bactérie (revue dans Kolodner *et al.,* 1995 ; Kolodner, 1996 ; Kunkel, 1995 ; Modrich et Lahue, 1996).

SYSTÈME DE RÉPARATION DE BASES MÉSAPPARIÉES

IDENTIFICATION DES GÈNES DU SYSTÈME DE RÉPARATION DE BASES MÉSAPPARIÉES

Une forme de cancer du côlon héréditaire porte le nom d'hereditary nonpolyposis colon cancer (HNPCC). Une recherche systématique du génome a été effectuée pour trouver le ou les gènes associé(s) à ce cancer. La méthodologie de liaison génétique (linkage) à l'aide de marqueurs microsatellites a mené à l'identification du premier locus de susceptibilité d'HNPCC sur le chromosome 2p (Peltomaki, 1993). Les tumeurs de ces patients montraient une variation dans le profil allélique des microsatellites analysés (Aaltonen *et al.,* 1993). À noter, un phénotype similaire avait été observé chez la bactérie et la levure possédant des mutations au niveau de gènes codant pour des composantes du système de réparation de mésappariements (SRM) (Levinso et Gutman, 1987 ; Strand *et al.,* 1993). Le gène du chromosome 2p, maintenant connu comme *hMSH2,* est l'homologue humain du gène *MSH2* chez *S. cerevisiae* et *MutS* de *E. Coli* (Figure 5, pp. 14 ; Leach *et al.,* 1993 ; Fishel *et al.,* 1993 ; Parsons *et al.,* 1993). Chez l'humain, on connaît maintenant au moins 6 gènes différents codant pour des composantes du SRM : *hMSH2, hMSH3, hMSH6* (homologues de *MutS*), *hMLH1, hPMS1, hPMS2* (homologues de *MutL*). Cependant, chez les eukaryotes il ne semble pas y avoir d'homologue pour la protéine *MutH* (Figure 5, pp. 14 ; Fishel et Kolodner, 1995 ; Fishel et Wilson, 1997 ; Gradia *et al.,* 1997).

Figure 5. Familles MutS et MutL des gènes SRM chez la bactérie, la levure et l'humain



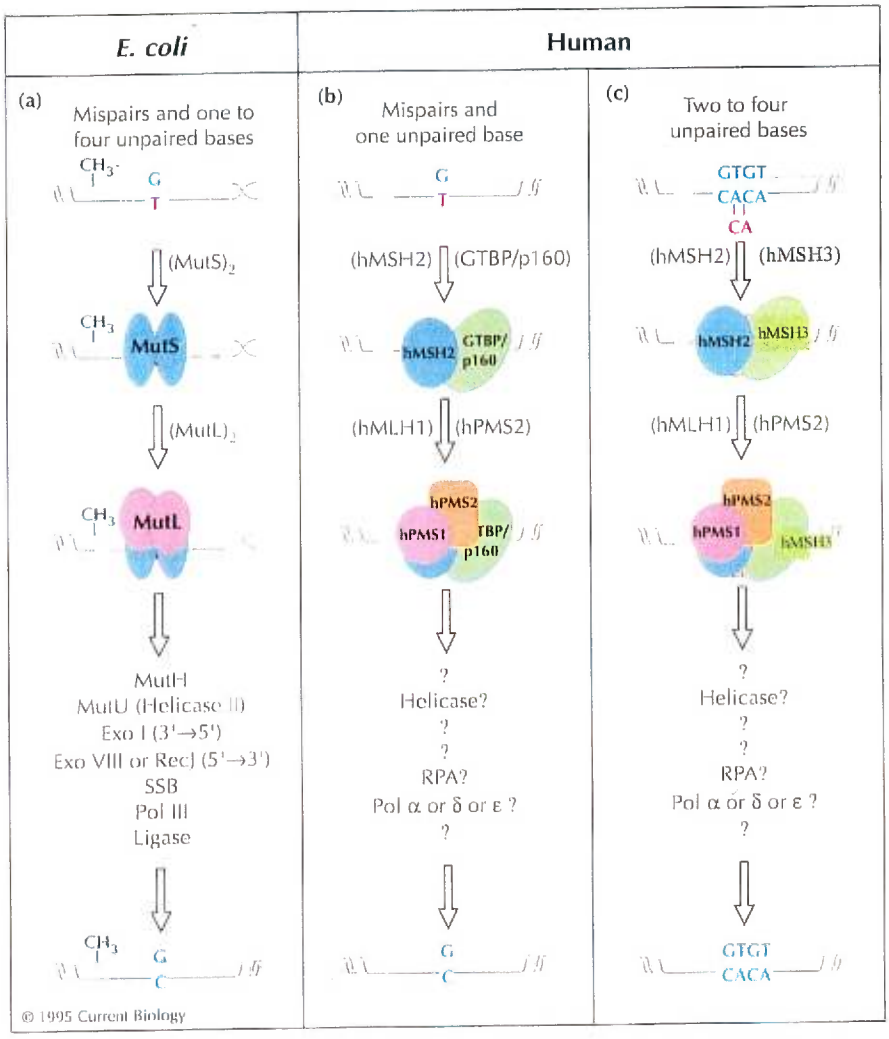
Modifié de Peltomaki et de la Chapelle, 1997.

Dans HNPCC, des mutations germinales sont présentes au niveau de 4 gènes SRM, soit les gènes *hMLH1, hMSH2, hPMS1* et *hPMS2* (Fishel *et al.,* 1993 ; Leach et *al.,* 1993 ; Bronner *et al.,* 1994 ; Papadopoulos *et al.,* 1994 ; Fishel et Kolodner, 1995). Les gènes les plus fréquemment mutés sont *hMLH1* et *hMSH2* tandis que les mutations pour *hPMS1* et *hPMS2* ne représentent qu'environ 10% des cas de HNPCC (Kolodner *et al.,* 1994 ; Nicolaides *et al.,* 1994 ; Liu *et al.,* 1996 ; Peltomaki *et al.,* 1997). Comme c'est le cas pour les gènes suppresseurs de tumeurs (Knudson, 1971), l'inactivation de ces gènes de réparation dans ce cancer se produit en deux étapes. Il y a un événement mutationnel qui est germinal (cancer héréditaire) et qui résulte en l'inactivation de l'un des deux allèles du gène. Ceci est suivi d'un deuxième événement mutationnel, celui-ci somatique, qui frappe le second allèle et mène à l'inactivation complète du gène SRM.

RÔLE DES GÈNES SRM

Chez *E. Coli* trois gènes, *MutS, MutL, MutH,* sont requis pour la correction d'erreurs commises lors de la réplication (Modrich, 1991). Dans ce système, la protéine *MutS* reconnaît le mésappariement et, suite à son interaction avec la protéine *MutL*, induit l'activité endonucléase de la protéine *MutH* (Figure 6, pp. 16 ; Modrich, 1991 ; Kunkel, 1995). Cette dernière coupe l'ADN sur le brin nouvellement synthétisé et permet la correction du mésappariement par l'entremise de nucléases, d'hélicases et de polymérases (Figure 6, pp. 16 ; Modrich, 1991 ; Kunkel, 1995). Chez les eukaryotes, la correction de mésappariements est plus complexe. Le mésappariement est premièrement reconnu par un complexe formé soit d'hMSH2 et d'hMSH3 (*hMutSβ*) ou bien du complexe formé d'hMSH2 et hMSH6 (*hMutSα*) (Figure 6, pp. 16 ; Kunkel, 1995 ; Drummond *et al.,* 1995 ;

Figure 6. Cheminements de réparation de bases mésappariées



Modifié de Kunkel, 1995.

Acharya *et al.*, 1996 ; Habraken *et al.*, 1996 ; Marsischky *et al.*, 1996 ; Palombo *et al.*, 1996). La protéine hMSH2 se lie aux mésappariements (Fishel *et al.*, 1994 a,b ; Mello *et al.*, 1996), alors que la spécificité et l'affinité de cette reconnaissance est amplifiée par son association avec hMSH3 ou hMSH6 (Drummond *et al.*, 1995 ; Acharya *et al.*, 1996 ; Palombo *et al.*, 1996). L'existence de ces deux types de complexes s'expliquerait par une spécificité de reconnaissance de mésappariements propre à chacun : MSH2-MSH6 reconnaît de simples mésappariements de bases et de petites insertions/délétions (Marsischky *et al.*, 1996 ; Sia et *al.*, 1997), alors que le complexe MSH2-MSH3 participerait à la réparation de mésappariements d'insertions/délétions de grandes tailles (Marsischky *et al.*, 1996 ; Sia *et al.*, 1997).

Le processus de réparation se poursuit par la reconnaissance de ce complexe protéines-ADN par un autre dimère formé d'hMLH1 et de hPMS2 (*hMutLα*) (Prolla *et al.*, 1994 ; Li et Modrich, 1995). Le rôle de *hPMS1* est peu connu. L'interaction de toutes ces protéines mène ensuite à la correction du mésappariement par l'entremise d'hélicases et de polymérases dont les identités ne sont pas encore connues chez l'humain.

AUTRES RÔLES DES GÈNES SRM

Les gènes SRM participent à plusieurs processus cellulaires vitaux autres que la réparation d'erreurs de réplication. Ces gènes sont impliqués dans la réparation préférentielle du brin transcrit de gènes actifs (TCR). Chez l'humain, une déficience en activité TCR a été associée avec l'inactivation de certains gènes SRM (Mellon *et al.*, 1996). Il a aussi été observé que les gènes SRM participent à la médiation du G2 *checkpoint* dans le cycle cellulaire (Hawn *et al.*, 1995). Ces gènes contribuent à conserver l'intégrité des séquences

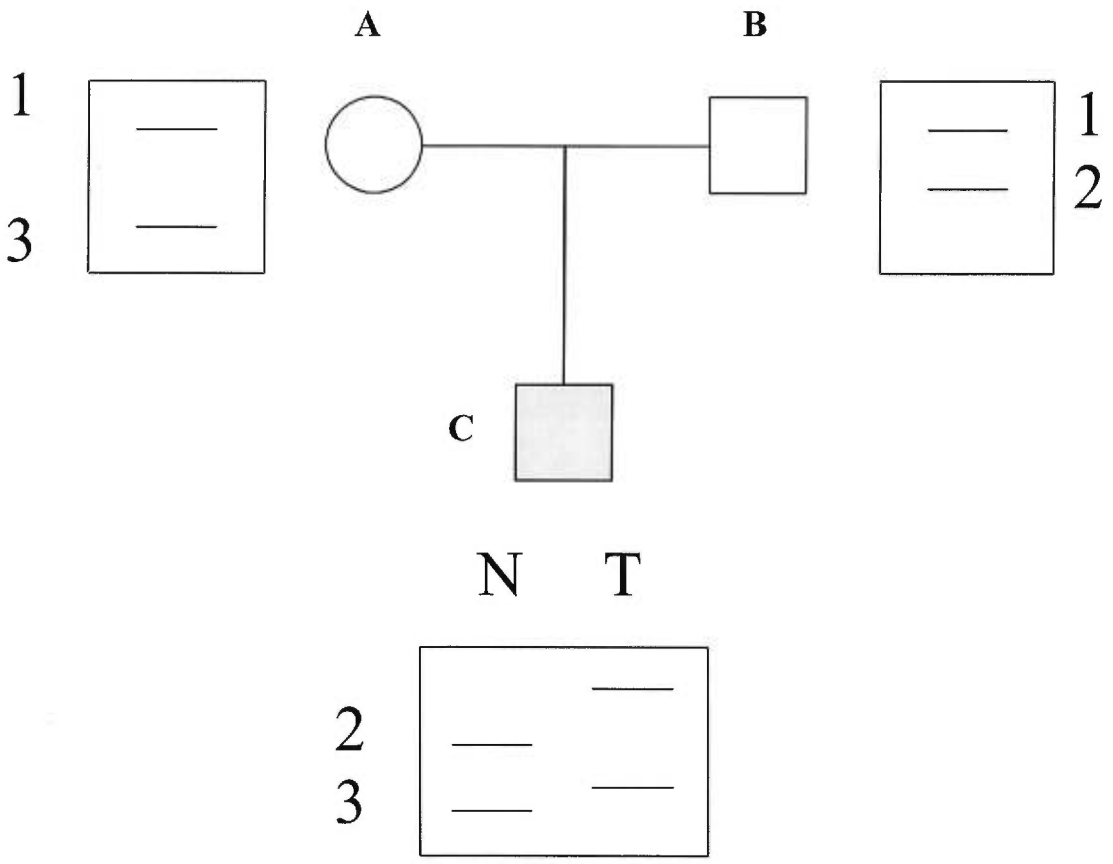
de l'ADN lors du processus de recombinaison homologue (deWind *et al.*, 1995). Des études de souris 'Knockout' de ces gènes indiquent que ceux-ci sont requis pour le bon déroulement du processus de méiose (Baker *et al.*, 1995). Finalement, ces gènes jouent un rôle dans la reconnaissance et/ou dans le cheminement de signalisation menant à l'induction de la mort cellulaire programmée ou apoptose (Kat *et al.*, 1993 ; Mu *et al.*, 1997).

PHÉNOTYPE MUTATEUR

Finalement, la 'signature' de l'inactivation des gènes SRM est l'augmentation du taux d'erreurs de réplication (RER) dans les cellules tumorales. Cette instabilité génétique (phénotype mutateur) affecte particulièrement les séquences de type microsatellites (Aaltonen *et al.*, 1993 ; Thibodeau *et al.*, 1993). L'instabilité des marqueurs microsatellites se présente comme une variation dans la migration électrophorétique d'un ou des deux allèles d'un locus donné (Figure 7, pp. 19). L'analyse de marqueurs microsatellites permet donc de classifier une tumeur comme étant soit RER+ ou RER- si aucune variation électrophorétique n'est observée aux loci étudiés. À l'aide de cette approche, un phénotype mutateur a été trouvé dans divers cancers héréditaires et sporadiques (Tableau III, pp. 20 ; Tableau IV, pp. 21). Près de 90% des cas d'HNPCC sont RER+ tandis que 15% des cancers sporadiques colorectaux le sont (de la Chapelle et Peltomaki, 1995). À noter, ces tumeurs RER+ possèdent des charactéristiques cliniques et pathologiques uniques en comparaison aux tumeurs RER- (de la Chapelle et Peltomaki, 1995). En plus des cas de cancer associés au HNPCC (tumeurs du côlon, de l'estomac, de l'endométrium et des ovaires, Lynch, 1993), la présence d'un phénotype mutateur a été observé dans plusieurs

cancers (Han *et al.*, 1993; Speicher *et al.*, 1995) dont le cancer sporadique du sein (Yee *et al.*, 1994; Kolodner et Alani, 1994 ; Karnik *et al.*, 1995; Paulson *et al.*, 1996). D'autres travaux ont suggéré la présence de ce phénomène pour les cas de cancer du poumon autres qu'à petites cellules (CPAPC) (Pifarre *et al.*, 1997) ainsi que pour les cas de cancer du poumon à petites cellules (Merlo *et al.*, 1994). Ces observations ont mené à l'hypothèse qu'une déficience dans la réparation de l'ADN par les gènes SRM serait aussi impliquée dans la tumorigenèse du sein et du poumon.

Figure 7. Exemple d'instabilité de microsatellites



A. Parent démontrant le profil allélique 1-3 pour un marqueur microsatellite.

B. Parent démontrant le profil allélique 2-3 pour le même marqueur microsatellite qu'en A.

C. Patient atteint d'une tumeur RER+ démontrant le profil allélique 2-3 au niveau de l'échantillon normal (N). Profil qui est conservé au niveau de l'échantillon tumoral (T) où une instabilité est reconnue par la variation au niveau de la migration électrophorétique.

Tableau III. Liste des cancers associés au HNPCC où un phénotype mutateur a été observé

- Cancer sporadique colorectal
- Adénome colorectal
- Syndrome de Muir-Torre
- Cancer associé aux colites ulcéreuses/dysplasies précancéreuses
- Néoplasies associées aux colites
- Carcinome gastrique
- Cancer de l'endométrium
- Cancer des ovaires
- Carcinome rénale
- Cancer de la vessie

Modifié de Speicher, 1995.

Tableau IV. Liste des cancers non-associés au HNPCC où un phénotype mutateur a été observé.

- Cancer du sein
- Cancer du poumon autre qu'à petites cellules
- Cancer du poumon à petites cellules
- Tumeurs des cellules germinales masculines
- Cancer du foie
- Cancer du cervix
- Sarcome de tissus mous
- Cancers primaires multiples
- Cancer du cerveau
- Adénocarcinome de l'oeosophage associé au syndrome de Barrett
- Cancer du pancréas

Modifié de Speicher, 1995.

HYPOTHÈSE

Les gènes SRM jouent un rôle important dans le développement des cancers sporadiques.

Pour vérifier cette hypothèse, nous proposons d'étudier les cancers sporadiques du sein, du poumon et du côlon.

OBJECTIFS

1- Déterminer, par l'analyse de marqueurs microsatellites, la présence d'un phénotype mutateur dans les cellules tumorales.

2- Détecter, à l'aide de marqueurs microsatellites, la présence de pertes alléliques au niveau des régions associées aux gènes SRM, *hMLH1*, *hMSH2*, *hMSH3*, *hMSH6*, *hPMS1* et *hPMS2*.

3- Examiner la relation entre l'expression d'un phénotype mutateur et la présence de pertes alléliques chez les cancers sporadiques étudiés.

4- Explorer l'implication d'autres mécanismes d'inactivation des gènes SRM.

FREQUENT LOSS of HETEROZYGOSITY at the DNA MISMATCH-REPAIR LOCI *hMLH1* and *hMSH3* in SPORADIC BREAST CANCER

Nadia Benachenhou[1], Sébastien Guiral[1], Izabella Gorska-Flipot[2], Damian Labuda[1,3] and Daniel Sinnett[1,3]

[1]Division of Hemato-Oncology, Charles Bruneau Cancer Center, Research Center, Ste-Justine Hospital, 3175 Côte Ste-Catherine Road, Montreal (Québec) H3T 1C5;

[2]Division of Pathology, Montreal Hôtel-Dieu Hospital;

[3]Department of Pediatrics, University of Montreal

Running title: LOH at mismatch-repair loci in breast cancer.

Corresponding author:

Daniel Sinnett

Centre de Cancérologie Charles Bruneau

Research Center, Sainte-Justine Hospital

3175 Côte Ste-Catherine, Montréal (Québec) H3T 1C5, Canada

Tel: (514) 345-4931, ext. 2990/Fax: (514) 345-4731

E-mail: sinnettd@ere.umontreal

SUMMARY

To study the involvement of DNA mismatch-repair genes in sporadic breast cancer, matched normal and tumoral DNA samples of 22 patients were analyzed for genetic instability and LOH with 42 microsatellites at or linked to *hMLH1* (3p21), *hMSH2* (2p16), *hMSH3* (5q11-q13), *hMSH6* (2p16), *hPMS1* (2q32), and *hPMS2* (7p22) loci. Chromosomal regions 3p21 and 5q11-q13 were found hemizygously deleted in 46% and 23% of patients, respectively. Half of the tumors which showed LOH at *hMLH1* were also deleted at *hMSH3*. The shortest regions of overlapping (SRO) deletions were delimited by markers *D3S1298* and *D3S1266* at 3p21 and by *D5S647* and *D5S418* at 5q11-q13. Currently, the genes *hMLH1* (3p21) and *hMSH3* (5q11-q13) are the only known candidates located within these regions. The consequence of these allelic losses is still unclear since none of the breast cancers examined displayed microsatellite instability, a hallmark of mismatch repair defect during replication error correction. We suggest that *hMLH1* and *hMSH3* could be involved in breast tumorigenesis through cellular functions other than replication error correction.

KEY WORDS: Mismatch-repair; *hMLH1*; *hMSH3*, LOH; breast cancer.

INTRODUCTION

Breast cancer is the most frequent neoplasia that affects women in the Western world. It is a heterogeneous disease, which displays a broad spectrum of clinical and pathological characteristics, and like most solid tumors, is thought to develop through the accumulation of genetic alterations leading to uncontrolled cellular growth. Loss of heterozygosity (LOH) studies in non-hereditary breast tumors have shown deletions at a frequency ranging from 20% to 50% in several chromosomal arms (reviewed in refs. Sato et al., 1990; Cornelisse et al., 1992; Bieche et al., 1995) suggesting the involvement of several tumor suppressor genes in breast carcinogenesis.

Recently, another type of gene, encoding components of the DNA mismatch-repair system, have been linked to hereditary non-polyposis colorectal cancer (HNPCC) (Leach et al., 1993; Fishel et al., 1993; Papadopoulos et al., 1994; Nicolaides et al., 1994; Bronner et al., 1994; Papadopoulos et al., 1995). These genes have been found mutated in HNPCC and presumably involved in certain sporadic forms of cancer (Leach et al., 1993; Papadopoulos et al., 1994; Nicolaides et al., 1994; Papadopoulos et al., 1995; Liu et al., 1995; Risinger et al., 1996). Their defects generally lead to a genome wide instability of microsatellites in tumoral cells referred to as the replication error (RER) phenotype. In addition to HNPCC, the RER phenotype was observed in a number of sporadic cancers (Han et al., 1993; Speicher et al., 1995) including breast cancer (Yee et al., 1994; Karnik et al., 1995; Paulson et al., 1996) thus suggesting that deficiency in DNA repair could be involved in breast carcinogenesis. Like suppressor genes (Knudson, 1971) mismatch-repair mutants are inherited as recessive traits that eventually become dominant due to somatic mutations inactivating the second allele. This second mutational step may be revealed as

LOH which was reported at the *hMLH1* locus in HNPCC patients (Hemminki et al., 1994) as well as in sporadic colorectal cancers (Tomlinson et al., 1996).

Based on these observations, we examined the involvement of mismatch-repair genes in sporadic breast cancer by microsatellite instability and LOH analyses. We have screened 22 primary breast carcinomas using 42 polymorphic microsatellites within or closely linked to *hMLH1*, *hMSH2*, *hMSH3*, *hMSH6*, *hPMS1*, and *hPMS2* loci. We found that *hMLH1* and *hMSH3* were frequently deleted in tumoral cells suggesting their possible involvement in sporadic breast cancer.

MATERIALS AND METHODS

DNA Samples. Matched tumoral and normal sample pairs were obtained from 22 breast carcinoma patients (ages 40 to 90; mean = 58.09, median = 60), including 10 metastatic cases, who underwent surgery at the Montreal Hôtel-Dieu Hospital. This is an unselected group of apparent sporadic cases with limited clinical information. Since family histories were unavailable, it was expected that if any, only 5 to 10% of the samples would be from patients with a familial form of the disease (Newman et al., 1988). DNA was isolated from fresh material by a standard procedure using digestion with proteinase K and phenol/chloroform extractions.

Microsatellite Analysis. Matched DNA sample pairs were genotyped by PCR at the 42 following highly polymorphic (62%-90% heterozygosity) microsatellite loci: on chromosome 3p14-p26 (*hMLH1*), *D3S1286, D3S1266, D3S1745, D3S1561, D3S1611,*

D3S1612, D3S1298, D3S1260, D3S3559, D3S3582, D3S3647, D3S1588, D3S1582, D3S1613, D3S1234, D3S1300 and *D3S1312*; on 5p14-q21 (*hMSH3*), *D5S416, D5S477, D5S651, D5S674, D5S426, D5S395, D5S418, D5S430, D5S491, D5S398, D5S431, D5S624, D5S427, D5S668, D5S647, D5S629, D5S428* and *D5S433;* on 2q32 (*hPMS1*), D2S318 and D2S118; on 2p16 (*hMSH2/hMSH6*), D2S391 and D2S288; on 7p22 (*hPMS2*), D7S531 and D7S517. The corresponding PCR primers were provided by Research Genetics, Inc. The chromosomal assignment of these microsatellites and genes was performed by integrating genetic, radiation hybrid and STS/YAC data from several sources (Gyapay et al., 1994; Hudson et al., 1995; Gemmill et al., 1993). Thirty amplification cycles of 1 min at 94°C, 1 min at 50°C-60°C and 1 min at 72°C were carried in 20 µl of 10 mM Tris-HCl (pH 8.3), 50mM KCl, 1.5 mM $MgCl_2$ containing 0.2 µM of each primer, 50 µM dNTPs, 1 µci of ^{32}P- αCTP (ICN; specific activity 3000 Ci/mmol), 5 ng of genomic DNA, and 0.4 U Taq DNA polymerase (BRL). The products were fractionated by denaturing electrophoresis in a 6% polyacrylamide gel, subsequently dried and autoradiographed. LOH was defined visually as the disappearance or significant reduction in the intensity of one allele in tumoral DNA compared to the normal DNA sample as described in Baccichet et al. (1997). Only informative (heterozygous) loci were considered for LOH frequency calculations.

Single-strand conformational polymorphism (SSCP) analysis. The typing of *hMLH1* exon 8 polymorphism by SSCP analysis using previously published oligonucleotides (Han et al., 1995) was performed as described in Zietkiewicz et al., 1992.

RESULTS

Our microsatellite analysis (Fig. 1) revealed LOH in 10 out of the 22 patients in at least one of the mismatch repair loci tested (Table 1).

Detection of LOH on chromosome 3p21. Out of the 22 patients, 10 (46 %) exhibited LOH in at least one of the microsatellite markers located on chromosome 3p21. Patient 3 lost an allele at *D3S1745* and *D3S1561* but maintained heterozygosity at the distal neighboring locus *D3S1266*; patient 43 showed LOH at every marker distal to *D3S1611* and *D3S1612* but retained both alleles at *D3S1298* (Fig. 2). These results suggested that the shortest region of overlapping (SRO) deletions delimited by *D3S1298* and *D3S1266* included *hMLH1* at 3p21-p22 (Fig. 2). In addition to the intragenic *D3S1611* marker (Papadopoulos et al., 1994), we analyzed by SSCP a biallelic polymorphism in exon 8 of *hMLH1* to show hemizygous deletion in the 3 informative cases (not shown).

Several genes have been shown to be included in LOH regions on chromosome 3p (Fig. 2). We extended the allelotyping to investigate the possible involvement of the SCLC region which was shown to be homozygously deleted in small cell lung cancer cell lines (Daly et al., 1993), as well as the *FHIT* and *PTPRG* genes (Fig. 2). Among the 10 patients with LOH at *hMLH1*, four retained heterozygosity at proximal markers (Fig. 2). For instance, in patient 11, markers *D3S1298* through *D3S1286* revealed LOH, but at markers proximal to *D3S1260* both alleles were retained, thus excluding the SCLC region as well as *FHIT* and *PTPRG* as deletion targets. Similarly, these loci were excluded from the SRO in patient 43 (Fig. 2). The SCLC region delimited by markers *D3S1588* and *D3S1613* (Daly et al., 1993) was partly affected by LOH in patients 3 and 29. Particular

Figure 1. Examples of LOH and heterozygosity of the 3p21 and 5q11-q13 regions. Patients 9, 11, 25 and 35 analyzed with marker D3S1745 and patients 3, 5, 11 and 13 analyzed with marker D5S427; Arrowhead indicate the deleted alleles in tumors; (N) normal and tumoral (T) DNA.

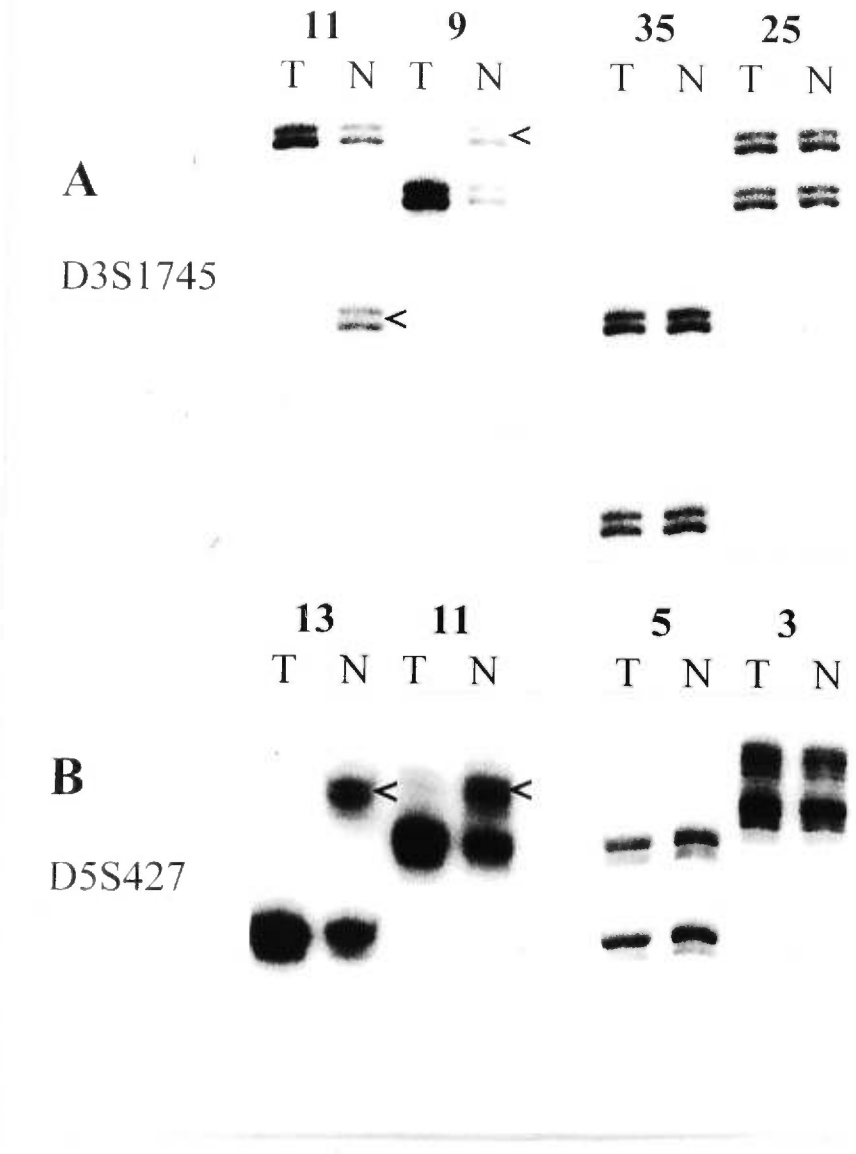
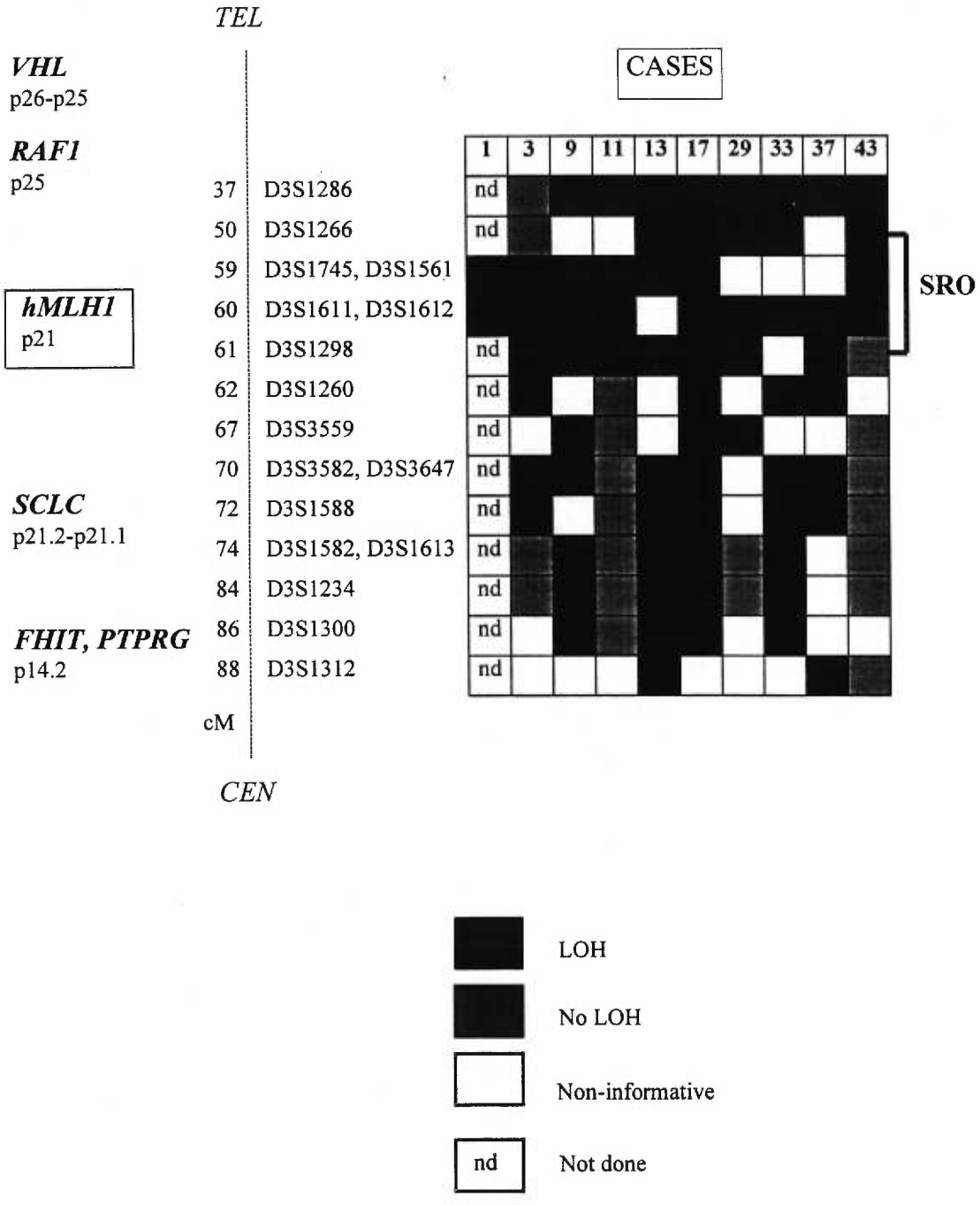


TABLE 1. SUMMARY OF THE LOH DATA FOR THE ANALYZED MISMATCH-REPAIR-RELATED CHROMOSOMAL REGIONS.

	DNA mismatch-repair-related region				
Cases	***hMLH1 (3p21)***	***hMSH3 (5q11-q13)***	***hPMS1 (2q32)***	***hMSH2 hMSH6(2p16)***	***hPMS2 (7p22)***
1	**LOH**	H	H	H	H
3	**LOH**	H	H	NI	H
5	H	H	H	H	H
7	H	H	NI	H	NI
9	**LOH**	**LOH**	**LOH**	**LOH**	**LOH**
11	**LOH**	**LOH**	**LOH**	H	H
13	**LOH**	**LOH**	H	H	H
15	H	H	H	H	H
17	**LOH**	H	H	H	H
19	H	H	H	H	H
21	H	H	NI	H	H
23	H	H	H	NI	H
25	H	H	H	H	H
27	H	H	H	H	H
29	**LOH**	**LOH**	H	H	H
31	H	H	H	H	H
33	**LOH**	H	H	H	H
35	H	H	H	H	H
37	**LOH**	**LOH**	H	H	H
39	H	H	H	H	H
41	H	H	H	H	H
43	**LOH**	H	H	H	H
LOH frequency	45.45%	22.7%	10%	5%	4.7%

LOH: Loss of heterozygosity; H: Heterozygote; NI: Non informative

Figure 2. LOH profiles around the 3p21 region and the shortest region of overlapping deletion (SRO).

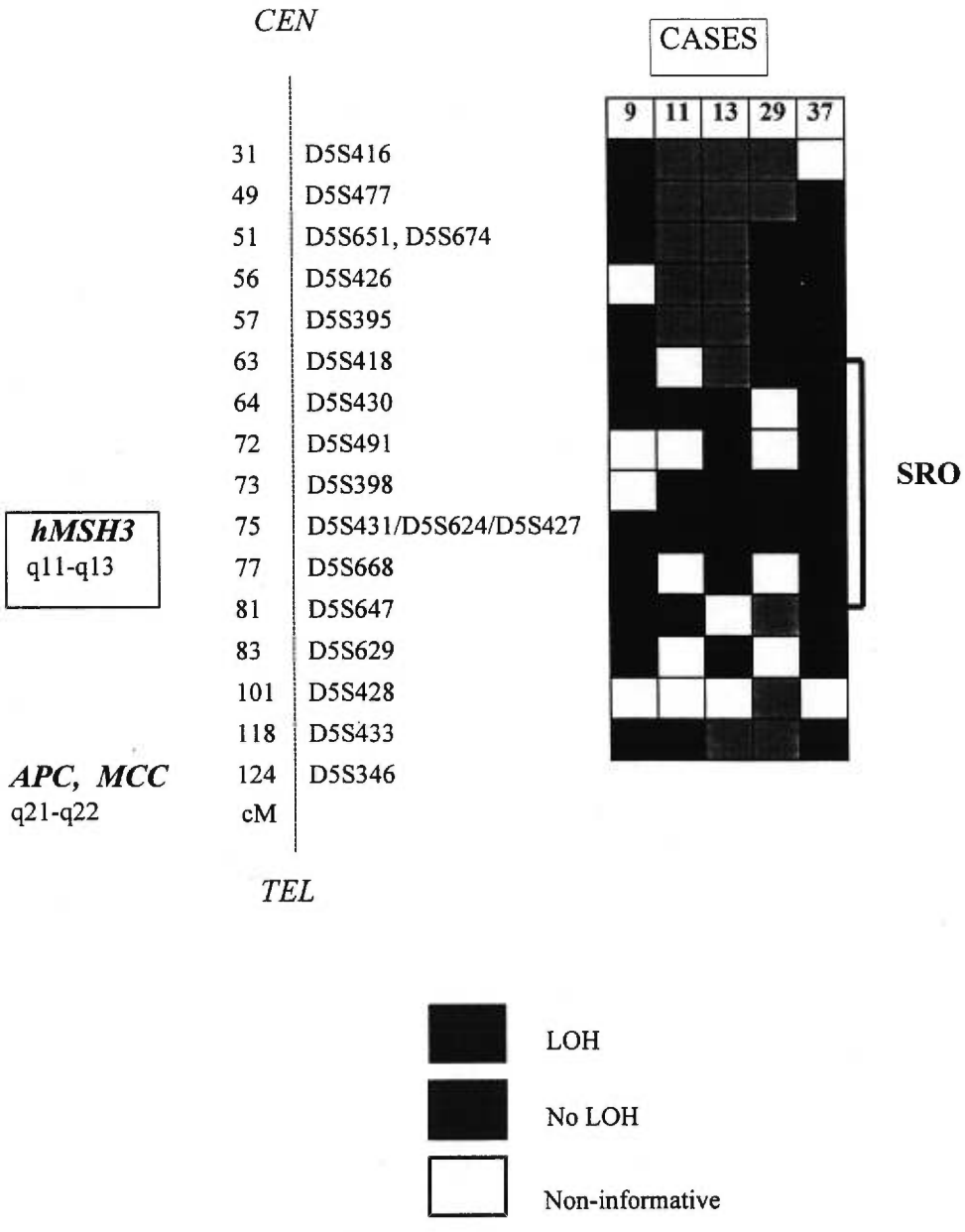


attention was placed on *FHIT* because abnormal transcription of this gene was reported in 30% of breast cancer patients (Negrini et al., 1996). Markers linked to the *FHIT* locus, including *D3S1300* which maps within intron 5 of the *FHIT* gene (Man et al., 1996), were deleted in 4 out of the 11 cases informative at this locus (36%), all also deleted for *hMLH1* (not shown). Therefore, 3p deletions represent two groups, one with small deletions affecting the *hMLH1* locus and another with larger deletions that include the *hMLH1*, SCLC and *FHIT* loci (Fig. 2).

Detection of LOH on chromosome 5q11-q13. As shown in Fig. 3, five out of the 22 informative cases (23%) were hemizygously deleted at one or more marker loci tightly linked to the *hMSH3* locus. All of these patients with LOH at 5q11-q13 were also deleted in the 3p21 region (Figs. 2 and 3). This non-random distribution of concomitant deletions was statistically significant (p~0.02, chi square test). Large deletions were seen in patients 9 and 37, whereas others exhibited restricted LOH: patient 13 with LOH at *D5S430* retained heterozygosity at every marker proximal to *D5S418*, whereas patient 29 was heterozygous at *D5S647*, thus delimiting the SRO between *D5S418* and *D5S647* at 5q11-q13 (Fig. 3). The tumor suppressor genes *APC* and *MCC* both located on chromosome 5q21 were excluded from deletions involving *hMSH3* in two of the patients (13 and 29) heterozygous for markers linked to *APC* and *MCC* region (Fig. 3). Thus, *hMSH3* is a good candidate as the target of 5q11-q13 deletions.

In contrast to *hMLH1* and *hMSH3*, we found only two patients that were affected by allelic deletions at other tested loci: 1 out of 21 informative cases at *hPMS2*, 1/20 at *hMSH2/hMSH6* and 2/20 at *hPMS1* (Table 1), including patient 9 who displayed LOH at all investigated loci. The low rate of allelic losses (5-10%) affecting the chromosomes

Figure 3. LOH profiles around the 5q11-q13 region and the shortest region of overlapping deletion (SRO).



2p16, 2q31-q33 and 7p15-pter may reflect the baseline frequency of LOH in breast cancer (Chen et al., 1992).

Microsatellite instability. None of the 22 tumors displayed the RER phenotype as judged by the absence of instability at the 42 microsatellites tested (a total of 572 independent comparisons). Although our markers were already shown sensitive to detect RER in sporadic colorectal cancers (Benachenhou et al., 1998a), we additionally examined two markers *GGAA4D07* and *GGAA2E02* recently reported unstable in 30% (11/37) and 41% (15/37) of breast cancer patients, respectively (Paulson et al., 1996). These 2 markers did not reveal any instability in the 22 tumors examined here (data not shown). Our findings were thus consistent with studies of two large cohorts of breast cancer patients that failed to detect significant level of microsatellite instability (Wooster et al., 1994; Lothe et al., 1993).

DISCUSSION

The activation of oncogenes, the loss or inactivation of repressor genes and impaired mismatch-repair function are known to be involved in the development of solid tumors. Defects in DNA mismatch repair genes lead to replication errors revealed as instability in microsatellite markers (Leach et al., 1993; Papadopoulos et al., 1994; Bronner et al., 1994). A proposal that deficient DNA repair was a predisposing factor in sporadic breast cancer (Helzlsouer et al., 1996; Parshad et al., 1996) was promoted by reports of microsatellite instability in breast tumors (Yee et al., 1994; Karnik et al., 1995; Paulson et

al., 1996). By allelotyping the mismatch repair genes *hMLH1*, *hMSH2*, *hMSH3*, *hMSH6*, *hPMS1*, and *hPMS2* we have shown that 46% and 23% of the breast tumors tested were affected by allelic losses at *hMLH1* and *hMSH3*, respectively. Since none of the tumor tissues were microdissected these figures should be considered conservative since some allelic losses could have been masked by contaminating genetic material of normal cells. Other alterations such as small deletions, point mutations, gene rearrangements, or DNA methylation, if they also contribute to inactivation of these loci could escape detection by our approach. Further studies are required to explore these possibilities.

Interstitial deletion of chromosome 3p is one of the most common genetic rearrangements observed in tumor cells (Pandis et al., 1993). The region 3p14-p23 has been shown to be deleted in small cell lung carcinomas (Petersen et al., 1997), non small cell lung carcinomas (Benachenhou et al., 1998b) renal cell carcinomas (Foster et al., 1994) and uterine cervical carcinomas (Kohno et al., 1993). In breast cancer, LOH frequencies ranging from 30-47% were observed at two separate regions, 3p13-p14 and 3p21-p25 (Chen et al., 1994) or 3p14.3-p21.1 and 3p24.3-p25.1 (Matsumoto et al., 1997), suggesting the involvement of several tumor suppressor genes. *PTPRG*, a protein-tyrosine phosphatase gene, and *FHIT* that encodes the human diadenosine triphosphate hydrolase (Barnes et al., 1996), both localised within the 3p13-p14 region, are potential candidates as targets of deletions in primary breast tumors (LaForgia et al., 1991; Ohta et al., 1996; Negrini et al. 1996). Furthermore, a 3p21.3 region (SCLC) was shown to be homozygously deleted in SCLC cell lines (Daly et al., 1993). Our allelotyping data narrow down the critically deleted region on chromosome 3p21 (Fig. 2) to an interval delimited by markers *D3S1298* and *D3S1266* thus excluding SCLC, *FHIT* and *PTPRG* from the SRO (Fig. 2). In another study we showed that the smallest region of overlapping deletions in non small

cell lung cancer patients was refined to a 1 cM interval between markers *D3S1561* and *D3S1612* (Benachenhou et al., 1998b). The only known candidate which remains in the deleted region is thus *hMLH1*. Does an hemizygous deletion of this gene promote cancer progression or is *hMLH1* only in linkage to a yet unidentified tumor suppressor gene ? This needs to be further investigated.

Deletions of chromosome 5q were previously reported in several tumor types including colorectal cancers (Solomon et al., 1987; Vogelstein et al., 1988; Ashton-Rickart et al., 1991), lung cancers (Ashton-Rickart et al., 1991; D'Amico et al., 1992; Benachenhou et al., 1998b), and eosophageal cancers (Boynton et al., 1992). At least two known tumor suppressors: *APC* and *MCC* are localized within this region. Reports of 5q21 deletions in 18-29% of sporadic breast cancers suggested the involvement of *APC* and *MCC* (Medeiros et al., 1994; Thompson et al., 1993). Although some deletions on 5q overlap with the *APC/MCC* region, our results exclude these loci from the SRO in 40% of the 5q deleted patients. Delimited by *D5S418* and *D5S647* this SRO is 43.5 cM away from *APC* and *MCC* (Fig. 3). Allelic losses at 5q13.1-q21 in 33% of ovarian cancers (Tavassoli et al., 1996) as well as in 42% of NSCLC (Benachenhou et al., 1998b) also lie outside the *APC* containing region. Thus genes other than *APC/MCC* have to be considered as targets of 5q11-q13 deletions. *hMSH3* is a good candidate as the target of 5q11-q13 deletions, although the existence of an as yet unidentified adjacent gene cannot be ruled out.

None of the tumors analyzed in this study displayed microsatellite instability, a hallmark of a deficiency in the replication errors correction. Expecting a 30% incidence of instability as reported by Paulson et al., 1996, the probability of not detecting a single unstable tumor in our sample of 22 was as low as 0.0014 ($Po = e^{-0.3 \times 22}$). The absence of RER is however consistent with earlier reports indicating a virtual absence of

microsatellite instability in breast tumor cells (Han et al., 1993; Lothe et al., 1993; Wooster et al., 1994). Moreover, considering four studies (Han et al., 1993; Lothe et al., 1993; Wooster et al., 1994; this study) where no evidence of RER was obtained (i.e. no more than a single instability per sample), we estimated the average rate of somatic microsatellite mutations at 5×10^{-3} (12/2556), a frequency similar to the one from T-lymphocytes (3×10^{-3}) (Hackman et al., 1995). It is difficult to explain the variability in the reported prevalence of RER+ breast tumors ranging from 0 to 30%. Since the random sampling effect was rather unlikely, this discrepancy could be related to sample stratification, to criteria used to define RER+ tumors (Dietmaier et al., 1997) and the nature of the markers used to reveal this phenotype (Arzimanoglou et al., 1998) or requires other explanations.

We are thus left with patients associated with hemizygous deletions at two mismatch-repair loci and no RER. If the non-deleted *hMLH1* and *hMSH3* alleles are still active this could explain the absence of a RER phenotype. At this point, it is difficult to decide whether hemizygous deletions of *hMLH1* and *hMSH3* genes promote breast tumorgenesis or whether LOHs at these loci only indicate linkage with as yet unknown tumor suppressor loci. But, concomitant deletions of *hMSH3* and *hMLH1* in a number of patients raise unanswered questions about their relationship. *hMSH2* and *hMSH6* or *hMSH3* proteins bind the mismatch as heterodimers called respectively *hMutSα* and *hMutS β* (Drummond et al., 1995; Palombo et al., 1996), which are then recognised by the heterodimer *hMutLα* composed of *hMLH1/hPMS2*. Therefore, a gene dosage effect affecting the stoichiometry and the activity of the heteromolecular mismatch-repair complex may be sufficient to promote cancer by impairing functions other than the correction of replication errors. Mismatch-repair proteins are involved in a variety of vital

cellular processes, including homologous recombination (Jones et al., 1987; deWind et al., 1995), mediation of the G2 checkpoint (Hawn et al., 1995), transcription-coupled nucleotide excision repair (Mellon et al., 1996) as well as in the recognition of DNA damage and in apoptosis (Kat et al., 1993; Mu et al., 1997). Interestingly, the influence of environmental factors on the genome stability in cells defective in nucleotide excision repair and mismatch-repair could be substantial. In this regard it has been recently demonstrated that homozygous as well as heterozygous *hMSH2* mutant mammalian cells have a propensity to accumulate potentially mutagenic oxidative DNA damage (DeWeese et al., 1997) that may promote the development of cancer through defects in genes sensitive to exogenous factors (Mellon et al., 1996; DeWeese et al., 1997). In addition, a heterozygous mutation in *hMLH1* in a human-derived cancer cell line was shown to significantly reduce transcription-coupled repair involved in selective removal of DNA damage from the transcribed strands of active genes (Mellon et al., 1996b), pointing to the possibility that allelic deletion of *hMLH1* and/or *hMSH3* could have the same effect. We propose that a subtle defect in the repair of DNA damage, which is less likely to be lethal to the carrying cells, could have an even more profound impact on tumorigenesis, thus placing individuals at increased cancer risk.

In conclusion, our allelotyping analysis of sporadic breast carcinomas demonstrated that two DNA mismatch repair loci, *hMLH1* and *hMSH3*, are frequently affected by LOH at chromosomal regions 3p21 and 5q11-q13 respectively. We suggest that *hMLH1* and *hMSH3* deletion could promote cancer progression through a dosage effect affecting cellular functions other than replication errors correction. Whether or not *hMLH1* and *hMSH3* are real targets of the deletions is still under investigation but the identification of genes with suppressor activity for malignancy at 3p21 and 5q11-q13 is extremely

important considering the frequency of LOH at these regions in several major forms of cancer.

REFERENCES

Arzimanoglou II, Gilbert F, Barber HRK (1998) Microsatellite instability in human solid tumors. *Cancer* **82**:1808-1820.

Ashton-Rickart PG, Wyllie AH, Bird CC, Dunlop MG, Steel CM, Morris RG, Piris J, Romanowski P, Wood R, White R, Nakamura Y (1991) MCC, a candidate familial polyposis gene in 5q21 shows frequent allele loss in colorectal and lung cancer. *Oncogene* **6**:1881-1886

Baccichet A, Qualman SA, Sinnett D (1997) Allelic loss in childhood acute lymphoblastic leukemia. *Leukemia Res.* **21(9)**:817-823

Barnes LD, Garrison PN, Siprashvili Z, Guranowski A, Robinson AK, Ingram SW, Croce CM, Ohta M, Huebner K (1996) Fhit, a putative tumor suppressor in humans, is a dinucleoside 5', 5'''-P1,P3 triphosphate hydrolase. *Biochemistry* **35**:11529-11535

Benachenhou N, Guiral S, Gorska-Flipot I, Michalski R, Labuda D, Sinnett D (1998a) Allelic losses and DNA methylation at DNA mismatch-repair loci in sporadic colorectal cancer (submitted)

Benachenhou N, Guiral S, Gorska-Flipot I, Labuda D, Sinnett D (1998b) High resolution deletion mapping reveals frequent allelic losses at the DNA mismatch-repair loci in *hMLH1* and *hMSH3* in non-small cell lung cancer. *Int. J. Cancer* **77**: 173-180

Bièche I, Lidereau R (1995) Genetic alterations in breast cancer. *Genes Chromosom. Cancer* **14**: 227-251

Boynton RF, Blount PL, Yin J, Brown VL, Huang Y, Tong Y, McDaniel T, Newkirk C, Resau JH (1992) Loss of heterozygosity involving the APC and MCC genetic loci occurs in the majority of human esophageal cancers. *Proc. Natl. Acad. Sci. USA* **89**:3385-3388

Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A, Tannergard P, Bollag RJ, Godwin AR, Ward DC, Nordenskjold M, Fishel R, Kolodner R, Liskay RM (1994) Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature* **368**:258-261

Chen LC, Kurisu W, Ljung BM, Goldman ES, Moore DJr, Smith HS (1992) Heterogeneity for allelic loss in human breast cancer. *J. Natl. Cancer Inst.* **84**:506-510

Chen LC, Matsumura K, Deng G, Kurisu W, Ljung BM, Lerman MI, Waldman FM, Smith HS (1994) Deletion of two separate regions on chromosome 3p in breast cancers. *Cancer Res.* **54**:3021-3024

Cornelisse CJ, Kuipers-Dijkshoorn N, van Vliet M, Hermans J, Devilee P (1992) Fractional allelic imbalance in human breast cancer increases with tetraploidization and chromosome loss. *Int. J. Cancer* **50**:544-548

Daly MC, Xiang RH, Buchhagen D, Hensel CH, Garcia DK, Killary AM, Minna J, Naylor SL (1993) A homozygous deletion on chromosome 3 in a small cell lung cancer cell line correlates with a region of tumor suppressor activity. *Oncogene* **8**:1721-1729

D'amico D, Carbone DP, Johnson BE, Meltzer J, Minna JD (1992) Polymorphic sites within the MCC and APC Loci reveal very frequent loss of heterozygosity in human small cell lung cancer. *Cancer Res.* **52**:1996-1999

deWind N, Dekker M, Berns A, Radman M, te Riele H (1995) Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell* **82**:321-330

DeWeese TL, Bucci JM, Larrier NA, Cutler RG, te Riele H, Nelson WG (1997) Tolerance of oxidative DNA damage results from disruption of *Msh2* alleles: implications for carcinogenesis. Proceedings, 88th Annual Meeting American Association for Cancer Research, vol. 38

Dietmaier W, Wallinger S, Bocker T, Kullmann F, Fishel R, Rüschoff J (1997) Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. *Cancer Res.* **57**:4749-4756

Drummond JT, Li GM, Longley MJ, Modrich P (1995) Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. *Science* **268**:1909-1912

Fishel R, Lescoe MK, Rao MRS, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* **75**:1027-1038

Foster K, Crossey PA, Cairns P, Hetherington JW, Richards FM, Jones MH, Bentley E, Affara NA, Ferguson-Smith MA, Maher ER (1994) Molecular genetic investigation of sporadic renal cell carcinoma: analysis of allele loss on chromosomes 3p, 5q, 11p, 17 and 22. *Br. J. Cancer* **69**:230-234

Gemmill RM, Chumakov I, Scott P, Waggoner B, Rigault P, Cypser J, Chen Q, Weissenbach J, Gardiner K, Wang H, Pekarsky Y, Le Gall I, Le Paslier D, Guillou S, Li E, Robinson L, Hahner L, Todd S, Cohen D Drabkin HA (1995) A second-generation YAC contig map of human chromosome 3. *Nature* **377(Suppl)**:299-319

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, Bernardi G, Lathrop M, Weissenbach J (1994) The 1993-94 Genethon human genetic linkage map. *Nat. Genet.* 7:246-339

Hackman P, Gabbani G, Österholm A-M, Hellgren D, Lambert, B (1995) Spontaneous length variation in microsatellite DNA from human T-cell clones. *Genes Chromosom. Cancer* **14**:215-219

Han HJ, Yanagisawa A, Kato Y, Park JG, Nakamura Y (1993) Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. *Cancer Res.* **53**:5087-5089

Han HJ, Maruyama M, Baba S, Park JG, Nakamura Y. Genomic structure of human mismatch repair gene, *hMLH1*, and its mutation analysis in patients with hereditary non-polyposis colorectal cancer (HNPCC). *Hum. Mol. Genet.* **4**:237-242.

Hawn MT, Umar A, Carethers JM, Marra G, Kunkel TA, Boland CR, Koi M (1995) Evidence for a connection between the mismatch repair system and the G2 cell cycle checkpoint. *Cancer Res.* **55**:3721-3725

Helzlsouer KJ, Harris EL, Parshad R, Perry HR, Price FM, Sanford KK (1996) DNA repair proficiency: Potential susceptibility factor for breast cancer. *J. Natl. Cancer Inst.* **88**:754-755

Hemminki A, Peltomaki P, Mecklin JP, Jarvinen H, Salovaara R, Nystrom-Lahti M, de la Chapelle A, Aaltonen LA (1994) Loss of the wild-type *MLH1* gene is a feature of hereditary nonpolyposis colorectal cancer. *Nat. Genet.* **8**:405-410

Hudson TJ, Stein LD, Gerety SS, Ma J, Castle AB, Silva J, Slonim DK, Baptista R, Kruglyak L, Xu S-H, Hu X, Colbert AME, Rosenberg C, Reeve-Daly MP, Rozen S, Hui L, Wu X, Vestergaard C, Wilson KM, Bae JS, Maitra S, Ganiatsas S, Evans CA, DeAngelis MM, Ingalls KA, Nahf RW, Horton Jr, LT, Anderson MO, Collymore AJ, Ye W, Kouyoumjian V, Zemsteva IS, Tam J, Devine R, Courtney DF, Renaud MT, Nguyen H, O'Connor TJ, Fizames C, Fauré S, Gyapay G, Dib C, Morissette J, Orlin JB, Birren BW, Goodman N, Weissenbach J, Hawkins TL, Foote S, Page DC Lander ES (1995) An STS-based map of the human genome. *Science* **270**:1945-1954

Jones M, Wagner R, Radman M (1987) Mismatch repair and recombination in *E. coli. Cell* **50**:621-626

Karnik P, Plummer S, Casey G, Myles J, Tubbs R, Crowe J, Williams BR (1995) Microsatellite instability at a single locus (*D11S988*) on chromosome 11p15.5 as a late event in mammary tumorigenesis. *Hum. Mol. Genet.* **4**:1889-1894

Kat A, Thilly WG, Fang WH, Longley MJ, Li GM, Modrich P (1993) An alkylation-tolerant, mutator human cell line is deficient in strand-specific mismatch repair. *Proc. Natl. Acad. Sci. USA* **90**:6424-6428

Knudson AG Jr (1971) Mutation and cancer: statistical study of retinoblastoma. *Proc. Natl. Acad. Med.* **68**:820-823

Kohno T, Takayama H, Hamaguchi M, Takano H, Yamaguchi N, Tsuda H, Hirohashi S, Vissing H, Shimizu M, Oshimura M (1993) Deletion mapping of chromosome 3p in human uterine cervical cancer. *Oncogene* **8**:1825-1832

LaForgia SK, Morse B, Levy J, Barnea G, Li F, Cannizarro LA, Nowell PC, Glick J, Boghosian-Sell L, Weston A, Harris CC, Drabkin H, Patterson D, Croce CM, Schlessinger J, Huebner K (1991) Receptor protein-tyrosine phosphatase is a candidate tumor suppressor gene at human chromosome region 3p21. *Proc. Natl. Acad. Sci. Usa* **88**:5036-5040

Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, Peltomaki P, Sistonen P, Aaltonen LA, Nystrom-Lahti M, Guan XY, Zhang J, Meltzer PS, Yu JW, Kao FT, Chen DJ, Cerosaletti KM, Fournier REK, Todd S, Lewis T, Leach RJ, Naylor SL, Weissenbach J, Mecklin JP, Järvinen H, Petersen GM, Hamilton SR, Green J, Jass J, Watson P, Lynch TT, Trent JM, de la Chapelle A, Kinzler KW, Vogelstein B (1993) Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* **75**:1215-1225

Liu B, Nicolaides NC, Markowitz S, Willson JK, Parsons RE, Jen J, Papadopolous N, Peltomaki P, de la Chapelle A, Hamilton SR, Kinzler KW, Vogelstein B (1995) Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. *Nat. Genet.* **9**:48-55

Lothe RA, Peltomaki P, Meling GI, Aaltonen LA, Nystrom-Lahti M, Pylkkanen L, Heimdal K, Andersen TI, Moller P, Rognum TO, Fossa SD, Haldorsen T, Langmark F, Brogger A, de la Chapelle A, Borresen AL (1993) Genomic instability in colorectal cancer: Relationship to clinicopathological variables and family history. *Cancer Res.* **53**:5849-5852

Man S, Ellis IO, Sibbering M, Blamey RW, Brook JD (1996) High levels of allele loss at the *FHIT* and *ATM* genes in non-comedo ductal carcinoma in situ and grade I tubular invasive breast cancers. *Cancer Res.* **56**:5484-5489

Matsumoto S, Kasumi F, Sakamoto G, Onda M, Nakamura Y, Emi M (1997) Detailed deletion mapping of chromosome arm 3p in breast cancers: a 2-cM region on 3p14.3-21.1 and a 5-cM region on 3p24.3-25.1 commonly deleted in tumors. *Genes Chromosom. Cancer* **20(3)**:268-274

Medeiros AC, Nagai MA, Neto MM, Brentani RR (1994) Loss of heterozygosity affecting the APC and MCC genetic loci in patients with primary breast carcinomas. *Cancer Epidemiol. Biomarkers Prev.* **3**:331-333

Mellon I, Rajpal DK, Koi M, Boland CR, Champe N (1996) Transcription-coupled deficiency and mutations in mismatch repair genes. *Science* **272**:557-560

Mu D, Turson M, Duckett DR, Drummond JT, Modrich P, Sanxcar A (1997) Recognition and repair of compound DNA lesions (base damage and mismatch) by human mismatch repair and excision repair systems. *Mol. Cell Biol.* **17**:760-769

Negrini M, Monaco C, Vorechovsky I, Ohta M, Druck T, Baffa R, Huebner K, Croce CM (1996) The *FHIT* gene at 3p14.2 is abnormal in breast carcinomas. *Cancer Res.* **56**:3173-3179

Newman B, Austin MA, Lee M, King MC (1988) Inheritance of human breast cancer: Evidence for autosomal dominant transmission in high risk families. *Proc. Natl. Acad. Sci. USA* **85**:3044-3048

Nicolaides NC, Papadopoulos N, Liu B, Wei YF, Carter KC, Ruben SM, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Dunlop MG, Hamilton SR, Peterson GM, de la Chapelle A, Vogelstein B Kinzler KW (1994) Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature* **371**:75-80

Ohta M, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, Siprashvili Z, Mori M, McCue P, Druck T, Croce CM Huebner K (1996) The human *FHIT* gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma associated translocation breakpoint, is abnormal in digestive tract cancers. *Cell* **84**:587-597

Palombo F, Iaccarino I, Nakajima E, Ikejima M, Shimada T, Jiricny J (1996) hMutSbeta, a heterodime of hMSH2 and hMSH3, binds to insertion/deletion loops in DNA. *Curr. Biol.* **6**:1181-1184

Pandis N, Jin Y, Limon J, Bardi G, Idvall I, Mandahl N, Mitelman F, Heim S (1993) Interstitial deletion of the short arm of chromosome 3 as a primary chromosome abnormality in carcinomas of the breast. Genes Chromosom. *Cancer* **6**:151-155

Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen C.A., Haseltine WA, Fleischmann, RD, Fraser CM, Adams MD, Venter, JC, Hamilton SR, Petersen GM, Watson P, Lynch HT, Peltomäki P, Mecklin JP, de la Chapelle A, Kinzler KW, Vogelstein B (1994) Mutation of a mutL homolog in hereditary colon cancer. *Science* **263**:1625-1629

Papadopoulos N, Nicolaides NC, Liu B, Parsons R, Lengauer C, Palombo F, D'Arrigo A, Markowitz, S, Willson JK, Kinzler KW, Jiricny J, Vogelstein B (1995) Mutations of GTBP in genetically unstable cells. *Science* **268**:1915-1917

Parshad R, Price FM, Bohr VA, Cowans KH, Zujewski JA, Sanford KK (1996) Deficient DNA repair capacity, a predisposing factor in breast cancer. *Br. J. Cancer* **74**:1-5

Paulson TJ, Wrught FA, Parker BA, Russack V, Walh GM (1996) Microsatellite instability correlates with reduced survival and poor disease prognosis in breast cancer. *Cancer Res.* **56**:4021-4026

Petersen I, Langreck H, Wolf G, Schwendel A, Psille R, Vogt P, Reichel MB, Ried T, Dietel M (1997) Small-cell lung cancer is characterized by a high incidence of deletions on chromosomes 3p, 4q, 5q, 10q, 13q and 17p. *Br. J. Cancer* **75**:79-86

Risinger JI, Umar A, Boyd J, Berchuck A, Kunkel A, Barrett JC (1996) Mutation in *MSH3* in endometrial cancer and evidence for its functional role in repair. *Nat. Genet.* **14**:102-105

Sato T, Tanigami A, Yamakawa K, Akiyama F, Kasumi F, Sakamoto G, Nakamura Y (1990) Allelotype of breast cancer: Cumulative allele losses promote tumor progression in primary breast cancer. *Cancer Res.* **50**:7184-7189

Solomon E, Voss R, Hall V, Bodmer WF, Jass JR, Jeffreys AJ, Lucibello FC, Patel I, Rider SH (1987) Chromosome 5 allele loss in human colorectal carcinomas. *Nature* **328**:616-619

Speicher MR (1995) Microsatellite instability in human cancer. *Oncol. Res.* 7:267-275

Tavassoli M, Steingrimsdottir H, Pierce E, Jiang X, Alagoz M, Farzaneh F, Campbell IG (1996) Loss of heterozygosity on chromosome 5q in ovarian cancer is frequently accompanied by TP53 mutation and identifies a tumor suppressor gene locus at 5q13.1-21. *Br. J. Cancer* **74**:115-119

Thompson AM, Morris RG, Wallace M, Wyllie M., Steel CM, Carter DC (1993) Allele loss from 5q21 (APC/MCC) and 18q21 (DCC) and DCC mRNA expression in breast cancer. *Br. J. Cancer* **68**:64-68

Tomlinson IP, Ilyas M, Bodmer WF (1996) Allele loss occurs frequently at *hMLH1*, but rarely at *hMSH2*, in sporadic colorectal cancers with microsatellite instability. *Br. J. Cancer* **74**:1514-1517

Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL (1988) Genetic alterations_during colorectal-tumor development. *N. Engl. J. Med.* **319**:525-532

Wooster R, Cleton-Jansen AM, Collins N, Mangion J, Cornelis RS, Cooper CS, Gusterson BA, Ponder BA, von Deimling A, Wiestler OD, Cornelisse CJ, Devilee P, Stratton MR (1994) Instability of short tandem repeats (microsatellites) in human cancers. *Nat. Genet.* **6**:152-156

Yee CJ, Roodi N, Verrier CS, Parl FF (1994) Microsatellite instability and loss of heterozygosity in breast cancer. *Cancer Res.* **54**:1641-1644

Zietkiewicz E, Sinnett D, Richer C, Mitchell G, Vanasse M, Labuda D (1992) Single-strand conformational polymorphism (SSCP): detection of useful polymorphisms at the dystrophin locus. *Hum. Genet.* **89**:453-456

Acknowledgements

N.B. has a fellowship from the Medical Research Council of Canada. S.G. has a studentship of the Fonds de la recherche en santé du Québec. D.S. is a scholar of the Fonds pour la Formation de Chercheurs et l'Aide à la Recherche. This work was supported by the Fondation Charles Bruneau.

Int. J. Cancer: 77, 173–180 (1998)

Publication of the International Union Against Cancer
Publication de l'Union Internationale Contre le Cancer

HIGH RESOLUTION DELETION MAPPING REVEALS FREQUENT ALLELIC LOSSES AT THE DNA MISMATCH REPAIR LOCI *hMLH1* AND *hMSH3* IN NON-SMALL CELL LUNG CANCER

Nadia BENACHENHOU[1,4], Sébastien GUIRAL[1,4], Izabella GORSKA-FLIPOT[2], Damian LABUDA[1,3] and Daniel SINNETT[1,3]*

[1]*Division of Hemato-Oncology, Charles Bruneau Cancer Center, Research Center, Sainte-Justine Hospital, Montreal, Canada*
[2]*Division of Pathology, Hôtel-Dieu Hospital, Montreal, Canada*
[3]*Department of Pediatrics, University of Montreal, Montreal, Canada*

To study the involvement of DNA mismatch repair genes in non-small cell lung cancer, matched normal and tumoral DNA samples from 31 patients were analyzed for both LOH and microsatellite instability with 34 markers at or linked to *hMLH1* (3p21), *hMSH2* (2p16), *hMSH3* (5q11-q13), *hMSH6* (2p16), *hPMS1* (2q32), and *hPMS2* (7p22) loci. Chromosomal regions 3p21 and 5q11-q13 were found to be hemizygously deleted in 55% and 42% of the patients, respectively. Sixty five percent of the patients deleted at *hMLH1* were also deleted at *hMSH3*. The shortest regions of overlap for 3p21 and 5q11-q13 deletions delimited by *D3S1611/D3S1612* and *D5S2107/D5S624*, respectively, were restricted to genetic distances of only 1 cM. Currently, the *hMLH1* (3p21) and *hMSH3* (5q11-q13) genes are the only known candidates located within these regions. The mutational analysis of *hMLH1* and *hMSH3* in hemizygously deleted patients led to the detection of 2 new polymorphisms in *hMSH3*. The consequence of these allelic losses remains unclear, but the lack of inactivating mutation might explain that replication error, the hallmark of mismatch repair genes inactivation in cancer cells, was quasi-absent in tumors. We suggest that *hMLH1* and *hMSH3* genes could be involved in lung tumorigenesis through dosage effect in cellular functions other than replication error correction.
***Int. J. Cancer* 77:173–180, 1998.**
© *1998 Wiley-Liss, Inc.*

Lung cancer is the leading cause of death from cancer worldwide with a survival rate as low as 10% despite intensive treatment. About 75% of all cases fall into the broad category of non-small cell lung cancer (NSCLC) which includes squamous cell carcinoma, adenocarcinoma and large cell carcinoma (Carney and de Leij, 1988). Lung carcinomas are characterized by multiple genetic alterations, which include the activation of oncogenes and the loss or inactivation of tumor suppressor genes (reviewed by Gazdar *et al.*, 1994). Mutations in the *p53* gene (Takahashi *et al.*, 1989), as well as deletions at chromosome 3p (Chiba *et al.*, 1990) appear to constitute the most common alterations involved in lung carcinogenesis. Frequent allelic losses affecting several chromosomal arms also strongly suggest the involvement of other yet unknown tumor suppressor genes (Petersen *et al.*, 1997).

Another type of genetic alteration, revealed as microsatellite instability in tumors (reviewed by Speicher, 1995), was reported in up to 45% of small cell lung cancers (Merlo *et al.*, 1994) and in up to 66% of NSCLC (Pifarre *et al.*, 1997). In hereditary non-polyposis colon cancer (HNPCC) this type of genetic instability underlies defects in replication errors correction due to inactivation of mismatch repair genes (reviewed by Peltomäki and de la Chapelle, 1997). Does the deficiency in DNA repair contribute to lung carcinogenesis? Like suppressor genes, inactivation of mismatch repair genes appears to occur in two steps. The first mutational event is either somatic (sporadic cancer) or germinal (hereditary cancer) followed by somatic mutations of the second allele. This second step may be revealed as LOH, as reported at the *hMLH1* locus in HNPCC patients (Hemminki *et al.*, 1994), as well as in sporadic colorectal cancer patients (Tomlinson *et al.*, 1996).

Based on these observations, we decided to investigate the involvement of mismatch repair genes in lung cancer by looking for both LOH and microsatellite instability at the corresponding genetic markers. Thirty-four highly polymorphic microsatellites within or closely linked to *hMLH1*, *hMSH2*, *hMSH3*, *hMSH6*, *hPMS1*, and *hPMS2* loci were analyzed in 31 matched normal/tumoral sample pairs from NSCLC patients. As we found allelic deletion of *hMLH1* and *hMSH3* in 55% and 42% of the cases respectively, we present a detailed deletion mapping of these genomic regions. Moreover, we report the results of *hMLH1* and/or *hMSH3* mutation screening in patients with deletions.

MATERIAL AND METHODS

Patients and DNA samples

This study involved 31 lung cancer patients (ages 32 to 78; mean = 58.8, median = 60.5) including 30 non-small cell lung tumors (7 carcinomas, 15 adenocarcinomas, 7 squamous cell carcinomas) and one node metastasis of a non-small cell lung tumor. Matched tumoral and normal DNA samples were isolated by a standard procedure using digestion with proteinase K and phenol/chloroform extractions.

Microsatellite analysis

Matched DNA sample pairs were genotyped by PCR at 34 highly polymorphic (62%–90% heterozygosity) microsatellite loci: chromosome 3p14-p26, *D3S1286, D3S1266, D3S1745, D3S1561, D3S1611, D3S1612, D3S1298, D3S1260, D3S3559, D3S3582, D3S3647, D3S1588, D3S1582, D3S1613, D3S1234, D3S1300* and *D3S1312*; 5p14–q21, *D5S418, D5S491, D5S398, D5S2107, D5S431, D5S624, D5S427, D5S668, D5S647, D5S629,* and *MCC;* 2q32, D2S318 and D2S118; 2p16, D2S391 and D2S288; 7p22, D7S531 and D7S517. The corresponding PCR primers were provided by Research Genetics (Huntsville, AL). The chromosomal assignment of these microsatellites was performed by integrating genetic, radiation hybrid and STS/YAC data from several sources (Genome Data Base; Généthon, Whitehead Institute for Biomedical Research/MIT Center for Genome Research, Cambridge, MA). Amplification conditions were 30 cycles of 1 min at 94°C, 1 min at 50°C–60°C and 1 min at 72°C in a 20 µl reaction containing 5 ng of genomic DNA, 0.2 µM of each primer, 50 µM dNTPs, 1 µci of α^{32}P- dCTP (ICN Biomedicals, Montreal, Canada); specific activity 3,000 Ci/mmol, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$ and 0.4 U Taq DNA polymerase (BRL, Burlington, Canada). The products were fractionated by electrophoresis on a 6% denaturing polyacrylamide gel, which was subsequently dried and autoradiographed. LOH was seen as

[4]NB and SG contributed equally to this work.

Grant sponsors: Fondation Charles Bruneau; Power Corporation-Fondation de l'Hôpital Sainte-Justine.

*Correspondence to: Charles Bruneau Cancer Center, Research Center, Sainte-Justine Hospital, 3175 Côte Ste-Catherine, Montréal (Québec) H3T 1C5, Canada. Fax: (514) 345–4731.
E-mail: sinnettd@ere.umontreal.ca

Received 29 September 1997; Revised 27 February 1998

TABLE I – CHARACTERISTICS OF THE PRIMERS USED FOR THE MUTATIONAL ANALYSIS OF *hMSH3* EXONS

hMSH3 exons	Sense primer sequence (5′ → 3′)	Antisense primer sequence (5′ → 3′)	Size of PCR product (bp)	Annealing temperature
Exon 1	GCTGCCATCCTTGCCCTG	GCCCAGTCCCAGACAGAACC	252 to 297[1]	55°C[2]
Exon 2	CAGGCCTTCTCAGAGTAGAG	AGAGCCATGACTGAGGAG	216	65°C
Exon 3	ACTATTGTTCTGTTTTCTTC	TTCTAGGTAACTGGATACTC	296	55°C
Exon 4	CTGGGAACTTATTATTGTGG	GAAAAATATAAAATAGTGCCTG	308	50°C
Exon 5	TGAACCAACATCACATAGGG	GGAGGAGAATCCCAATTTC	233	55°C
Exon 6	CATCTTTTATCCTTTGATAGC	GCCCTTGTATCATTTAAGAG	207	55°C
Exon 7	ATTAGGATTTAGAATTTAGC	AGATACCATGAACATCTAAC	243	55°C
Exon 8	TGTTTATGCTGTGTTATAGCC	AACCTAAGTTTCAGAATCGG	290	55°C
Exon 9	TGATAATGGATAAGTTATCTTTG	ATCATACGAATACCTTATCC	226	55°C
Exon 10	GCTAACAAGTTAATGGTTCTG	GTTCATAATATAAGCTCCCC	255	55°C
Exon 11	TTATGCACTTACATCTAGGC	CAACTAGCCCTTGTATTTG	154	55°C
Exon 12[3]	TGGTCTTTCTTAGACTGATATG	GCTCGAATTCCTTTTACC	140	55°C
Exon 13	CCTAACATATCTGATTATTGC	AGTGTCCTCAAGCTGAAG	228	55°C
Exon 14[3]	ACATTTTCATGTATCTTATGC	GGGTCTGGTACTTACTTGG	253	55°C
Exon 15	TGCTGTAATTAATAAACTTCG	AGAAAATAAAAGTAAGCTTGA	247	55°C
Exon 16	ACCACAATTTGGGGAAAG	ATGAGCCCAGTAGCACATAG	214	55°C
Exon 17	GCATTTCGGATTTTTTAC	TTCTGATAGCAAAAAGTCAG	221	55°C
Exon 18	AGGTTTAAGATATCAGTTTGC	TCTAAAGCACTGATAAAGAGG	201	55°C
Exon 19	AAGGCTATTTCCATGCCTAG	TTTGGGATGTTATCATCGAC	205	55°C
Exon 20	GTAATGTTTTGCCTAATGC	TCAAGAATATATGCAAGCTG	292	55°C
Exon 21	GTGAAGAGGAAAATCAAGG	ATACAGTTTACCGGATACTTAC	248	55°C
Exon 22	ATTCTGTCTTATTGCTTTAGG	TATTTGTACCAACTGCAGG	181	55°C
Exon 23	TCAGGCACAGTTTTGATC	CCTCTCTTTCTTGGACAAC	276	55°C
Exon 24	TAAGTAGTATTTGATTTTTCCC	GTCACACAAAGATAGGCTG	228	55°C

[1]9 bp repeat polymorphism.–[2]In presence of 8% formamide, 6% DMSO.–[3]Due to difficulties in obtaining specific PCR products for exons 12 and 14, primers show short overlaps with coding regions of exons 12 and 14, respectively.

disappearance or significant reduction in intensity of one allele in tumor DNA compared to the normal DNA sample. Only informative (heterozygous) loci were considered for LOH frequency calculations.

Single-strand conformation polymorphism (SSCP) and sequence analyses

Screening of *hMLH1* and *hMSH3* for mutation was performed by PCR-SSCP. Primer pairs listed in Table I for *hMSH3* analysis were designed to flank each of the 24 exons of *hMSH3*, based on the sequence published by Watanabe *et al.* (1996) Typing of *hMLH1* exon 8 polymorphism as well as screening *hMLH1* for mutations were performed using previously published oligonucleotides flanking the 19 exons of *hMLH1* (Han *et al.*,1995). Amplification conditions were performed as for microsatellites (see above) except for *hMSH3* exon 1 where 8% formamide and 6% DMSO were added to the PCR mix because of the GC richness of a 9 bp repeat (Nakajima *et al.*,1995). All SSCP variants were directly sequenced using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham, Arlington Heights, IL).

RESULTS

Our allelotyping analysis (Fig. 1) revealed LOH in 20 out of the 31 tumor samples in at least one of the markers tested (Table II).

Deletion mapping of the 3p21 chromosomal region

Out of the 31 patients, 17 (55%) exhibited LOH at informative markers of the 3p21 region (Table II and Fig. 2). To define the shortest region of overlapping (SRO) deletions, those deleted cases were further analyzed with 13 flanking markers (Fig. 2). The following patients were particularly informative: patients 1 and 13 lost an allele at *D3S1611* but maintained heterozygosity at the distal neighboring locus *D3S1561*; patients 1 and 39 retained both alleles at *D3S1612* but were hemizygously deleted at *D3S1611* and *D3S1561*, respectively (Fig. 2). These results delimited the SRO at 3p21–p22 to a genetic interval of 1 cM including *hMLH1*, between *D3S1561* and *D3S1612* (Fig. 2).

Chromosome 3p deletions in lung tumors have been shown to include several known genes (Fig. 2). Particular attention was placed on *FHIT/PTPRG* and the SCLC region between position 72

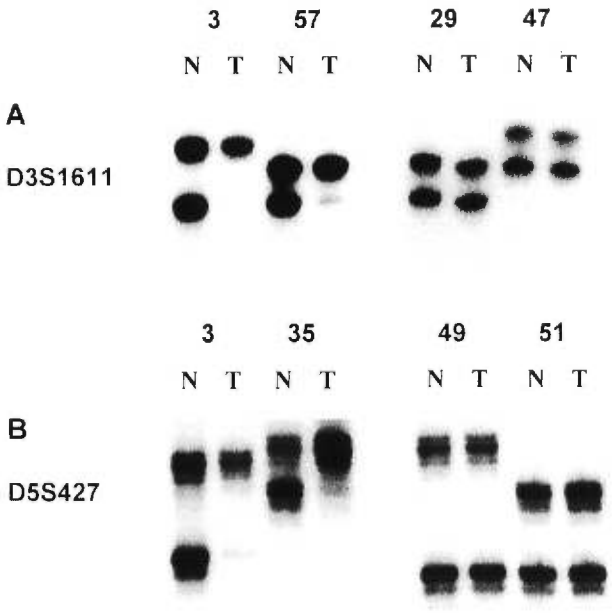


FIGURE 1 – Detection of LOH affecting the 3p21 and 5q11-q13 chromosomal regions. (*a*): patients 3, 57, 29 and 47 analyzed with marker *D3S1611*. (*b*): patients 3, 35, 49 and 51 analyzed with marker *D5S427*. (N) normal and tumoral (T) DNA.

cM–73 cM because abnormal transcription of *FHIT* (Sozzi *et al.*, 1996) and homozygous deletions of the SCLC region (Daly *et al.*, 1993) were reported. Our data allowed the delineation of a distinct deleted region (Fig. 2): among the 17 cases shown to be deleted at *hMLH1*, 4 were heterozygous at *FHIT* and *PTPRG*, and 3 at SCLC, thus excluding those loci from the SRO (Fig. 2). Only 9/21 informative cases (43%) were deleted at the markers flanking the SCLC region (*D3S1588* and *D3S1613*) and 9/21 informative cases (43%) were deleted at the marker *D3S1300* intragenic to *FHIT*. Our

TABLE II – LOH RESULTS FOR THE 6 ANALYZED MISMATCH-REPAIR LOCI

Cases[1]	*hMLH1* (3p21)	*hMSH2 MSH6* (2p16)	*hMSH3* (5q11-q13)	*hPMS1* (2q32)	*hPMS2* (7p22)
11c	H	H	LOH	H	H
21a	H	H	LOH	H	H
5a	LOH	H	LOH	H	H
31c	LOH	H	LOH	NI	H
35c	LOH	H	LOH	NI	H
41c	LOH	NI	LOH	H	H
53a	LOH	H	LOH	H	H
55c	LOH	H	LOH	H	NI
57a	LOH	H	LOH	H	H
59c	LOH	NI	LOH	H	NI
1c	LOH	H	H	H	H
51a	LOH	H	H	H	H
15a	LOH	H	H	H	H
23a	LOH	H	H	H	H
39a	LOH	LOH	H	H	H
13a	MI(LOH)	MI(NI)	MI(H)	H	MI(LOH)
3c	LOH	LOH	LOH	LOH	H
17m	LOH	H	LOH	LOH	LOH
43c	LOH	NI	LOH	LOH	LOH
33c	H	LOH	H	H	H
7c	H	H	H	H	H
9a	H	H	H	H	H
19c	H	H	H	H	H
25a	H	NI	H	H	H
27a	H	H	H	H	H
29c	H	H	H	H	H
45a	H	H	H	H	H
47a	H	H	H	H	NI
49c	H	H	H	H	H
61a	H	H	H	H	H
63a	H	H	H	H	H
LOH frequency	54.8%	11.5%	41.9%	10.3%	10.7%

[1]a: Adenocarcinoma; c: Carcinoma; m: Lymph node metastasis; LOH: loss of heterozygosity; H: heterozygote; NI: noninformative; MI: microsatellite instability.

results indicate that the most frequent 3p deletion (55%) includes *hMLH1*.

Deletion mapping of the 5q11-q13 chromosomal region

As shown in Figure 3, markers tightly linked to the *hMSH3* locus were hemizygously deleted in 13 out of the 31 tumors (42%), 11 of which were also deleted in the 3p21 region (Table II and Fig. 3). This non-random distribution of concomitant deletions was statistically significant ($P < 0.01$, Fisher's test). The extended allelotyping demonstrated heterozygosity at *D5S2107* in patient 17 and at *D5S624* in patients 11, 17, 21, 53 and 55, all hemizygously deleted at *D5S431*. The SRO at 5q11-q13 was thus delimited by markers *D5S2107* and *D5S624*, a genetic distance of 1 cM. In 5 cases a second distinct distal deleted region was also observed (Fig. 3). Of note, the *APC/MCC* loci were clearly excluded from the SRO encompassing *hMSH3* in patients 11, 17, 21, 53, 55 and 59. LOH at the *MCC* marker was observed in 6 out of the 14 informative cases (38%), all of which were also deleted at the *hMSH3* region. *hMSH3* is currently the only known candidate localized within the 1 cM commonly deleted region.

The low level of allelic losses (10–11%) affecting the chromosomes 2p16, 2q31–q33 and 7p15-pter (Table II) may reflect the background of LOH in cancer cells.

Microsatellite instability analysis

Each of the 31 pairs of tumoral and matching normal DNAs were analyzed for microsatellite instability with 19 to 34 markers. Only one tumor (3.2%) was found unstable at more than one microsatellite (22 out of 30). This patient was hemizygously deleted at *hMLH1* but no evidence of inactivating mutation in the remaining allele was obtained by SSCP analysis (see below), suggesting the possible involvement of another mismatch repair gene in the observed RER phenotype.

Our findings are consistent with earlier studies in 3 large cohorts of NSCLC (Peltomaki *et al.*, 1993; Fong *et al.*, 1995; Ryberg *et al.*, 1995) where similarly low levels of microsatellite instability were observed (Table III). Taken together, 540 NSCLC patients were analyzed at 92 loci in 8 studies (Pifarre *et al.*, 1997; Peltomäki *et al.*, 1993; Fong *et al.*, 1995; Ryberg *et al.*, 1995; Shridhar *et al.*, 1994; Wieland *et al.*, 1996; Mao *et al.*, 1994) including this one. For a total of 4,591 paired typings, 228 revealed instability (228/4591, 5%). Excluding 64 tumors (59%, 64/109) showing only a single unstable locus, we are left with 8% of the cases displaying microsatellite instability (Table III). Thus, in spite of frequent deletions involving *hMLH1* and *hMSH3*, replication errors appear to play a moderate role in non-small cell lung tumorigenesis.

Mutational analysis of hMLH1 and hMSH3 in patients with deletions

A total of 3 SSCP variants and one length polymorphism were identified in the patients hemizygously deleted at *hMLH1* ($n = 17$) and *hMSH3* ($n = 13$). Two of these have been previously observed: the *hMLH1* exon 8 polymorphism (Liu *et al.*, 1995), changing Ile219 to Val (ATC → GTC), was found in both the patients and normal controls (Table IV). The 9bp repeat in the *hMSH3* exon 1, previously shown to be polymorphic in Japanese patients (Nakajima *et al.*, 1995), was variable in size in our Caucasian population (Table IV). In addition, we have identified two novel mutations in *hMSH3*. Examples of SSCP findings are presented in Figure 4. One of these mutation caused an Arg to Gln change as a consequence of a CGG→CAG transition at codon 940 of *hMSH3* exon 21 (Fig. 4, Table IV). Of note, this position is part of a protein domain conserved in mice and in *E. coli* (Fig. 4). The other new missense mutation occurred at *hMSH3* codon 1036 (exon 23) as a consequence of a ACA → GCA transition (Fig. 4). All these variant sequences were present in both tumoral and normal DNA from more than one NSCLC patients and also in blood DNA of normal controls (Table IV) suggesting that these mutations represent common polymorphisms rather than pathogenic changes.

DISCUSSION

The activation of oncogenes, the loss or inactivation of suppressor genes and impaired mismatch repair function are known to be involved in the development of solid tumors. Defects in DNA mismatch repair genes lead to replication errors revealed as instability at microsatellite markers. The suggestion that reduced DNA repair capacity is a predisposing factor in lung cancer (Wei *et al.*, 1995) is supported by reports of frequent microsatellite instability in lung tumors (Pifarre *et al.*, 1997; Shridhar *et al.*, 1994; Wieland *et al.*, 1996). By allelotyping the mismatch repair genes *hMLH1*, *hMSH2*, *hMSH3*, *hMSH6*, *hPMS1*, and *hPMS2* we have shown that 55% and 42% of the NSCLC tested were affected by allelic losses at *hMLH1* and *hMSH3*, respectively. These figures should be considered conservative since some allelic losses could have been masked by contaminating genetic material from normal cells. Other alterations such as small deletions, point mutations, gene rearrangements, or DNA methylation, if they also contribute to inactivation of these loci could escape detection by our approach. Further studies are required to explore these possibilities.

Interstitial deletion of chromosome 3p is one of the most common genetic rearrangements observed in tumor cells (reviewed by Kok *et al.*, 1997). In lung cancer, LOH at 3p was reported in nearly 100% of SCLC (Kok *et al.*, 1987; Naylor *et al.*, 1987) and in more than 50% of NSCLC (Kok *et al.*, 1987; Rabbitts *et al.*, 1989). The finding of overlapping homozygous deletions in lung tumors derived cell lines (reviewed by Kok *et al.*, 1997), in addition to direct tumors (Roche *et al.*, 1996), strongly suggests the presence of one or more 3p tumor suppressor genes. In fact, tumor suppression activity has been demonstrated by several groups either for the entire chromosome 3 or for only a portion of it

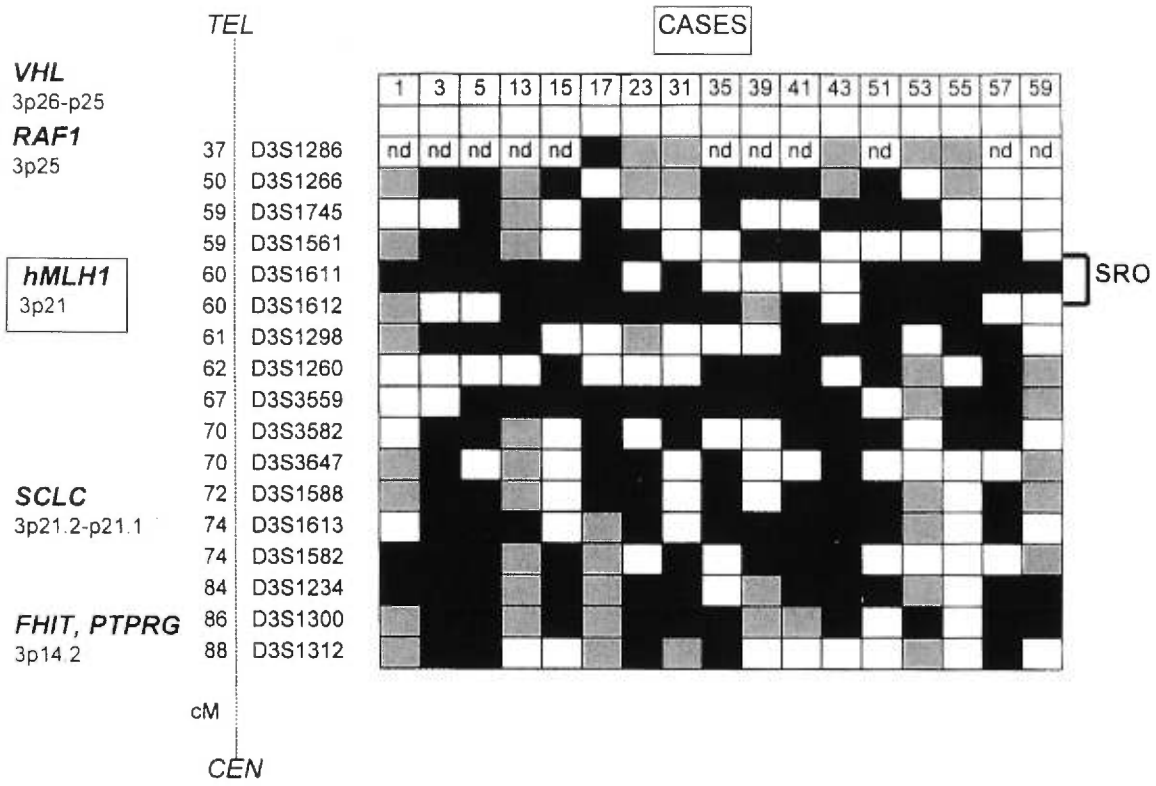


FIGURE 2 – LOH profiles around the 3p21 region and the shortest region of overlapping deletion (SRO).

(reviewed by Kok *et al.*, 1997). Our allelotyping data narrowed down the critically deleted region on chromosome 3p21 to an interval of only 1 cM delimited by markers *D3S1561* and *D3S1612* (Fig. 2), distinct from previously described deleted regions including *FHIT*. The only known candidate that remains in the deleted region is *hMLH1*. Does hemizygous deletions of this gene promote cancer progression or is *hMLH1* only in linkage to a yet unidentified tumor suppressor gene need to be investigated further.

Deletions of chromosome 5q have been previously reported in several tumor types including colorectal cancers (Solomon *et al.*, 1987), sporadic breast cancers (Medeiros *et al.*, 1994; Benachenhou *et al.*, 1998), esophageal cancers (Boynton *et al.*, 1992) and ovarian cancer (Tavassoli *et al.*, 1996). Allelic deletion of 2 known tumor suppressors present in this region, *APC* and *MCC*, was frequently observed in lung cancer (D'Amico *et al.*, 1992). Although some deletions on 5q overlap with the APC/MCC region (Fig. 3), our results exclude these loci from the SRO in 54% of the 5q deleted patients. Delimited by *D52107* and *D5S64*, a genetic distance of 1 cM, this SRO is 49 cM away from *APC* and *MCC* (Fig. 3). Allelic losses in 33% of ovarian cancers (Tavassoli *et al.*, 1996) and in 40% of 5q11–q13 deleted breast cancers (Benachenhou *et al.*, 1998) also lay outside APC containing region. Thus, genes other than *APC/MCC* have to be considered as targets of 5q11–q13 deletions. *hMSH3* is a good candidate as the target of 5q11–q13 deletions described here, although the existence of a yet unidentified adjacent gene cannot be ruled out.

Only one tumor (patient 13) displayed microsatellite instability, a hallmark of a deficiency in the correction of replication errors. The mutational analysis of this patient failed to reveal any mutation in the remaining *hMLH1* allele, which may suggest other mechanisms of *hMLH1* inactivation such as methylation of the promoter (Kane *et al.*, 1997), or that *hMLH1* is not responsible for the expression of the RER phenotype in our patient. The low frequency of RER (3%) seen in this study is consistent with previous reports indicating a virtual absence (Peltomäki *et al.*, 1993) or only a low level (Fong *et al.*, 1995; Ryberg *et al.*, 1995) of microsatellite instability at more than one locus in lung tumors. Indeed, when one considers the data on 540 NSCLC cases, only 5% (228/4591) of paired typings displayed instability (Table III). Actually only 45 out of 540 (8%) tumors were unstable at more than one locus with the proportion of unstable tumors ranging from 0 to 66% (Table III). Since the random sampling effect was rather unlikely, this discrepancy could be related to samples stratification, to criteria used to define RER^+ tumors or requires other explanations. The low average frequency of microsatellite instability in NSCLC from 8 available reports, including our study, reinforces our earlier conclusions that a deficiency in the correction of replication errors plays only a moderate role in non-small cell lung tumorigenesis.

We are thus left with frequent hemizygous deletions at 2 mismatch repair loci and no RER in most NSCLC cases. The mutational analysis of these patients led to the identification of 4 DNA variants including 2 known polymorphisms in exons 8 and 1 of *hMLH1* and *hMSH3*, respectively. The significance of the 2 other germline mutations in *hMSH3* that gave rise to amino-acid changes ($Arg_{940}Gln$ and $Thr_{1036}Ala$) remains unclear. The presence of all 4 variants in the normal population points to common polymorphisms rather than pathogenic changes. Therefore, the absence of inactivating mutation in the remaining *hMLH1* and *hMSH3* alleles in these tumors could explain the absence of a RER phenotype. At this point, it is difficult to decide whether hemizygous deletions of *hMLH1* and *hMSH3* genes promote lung tumorigenesis or whether LOHs at these loci only indicate linkage with yet unknown tumor suppressor loci. But, concomitant deletions of *hMSH3* and *hMLH1* in a number of patients raise questions about their possible interdependence. *hMSH2* and *hMSH6* or *hMSH3* proteins bind the mismatch as heterodimers called respectively *hMutS*α and

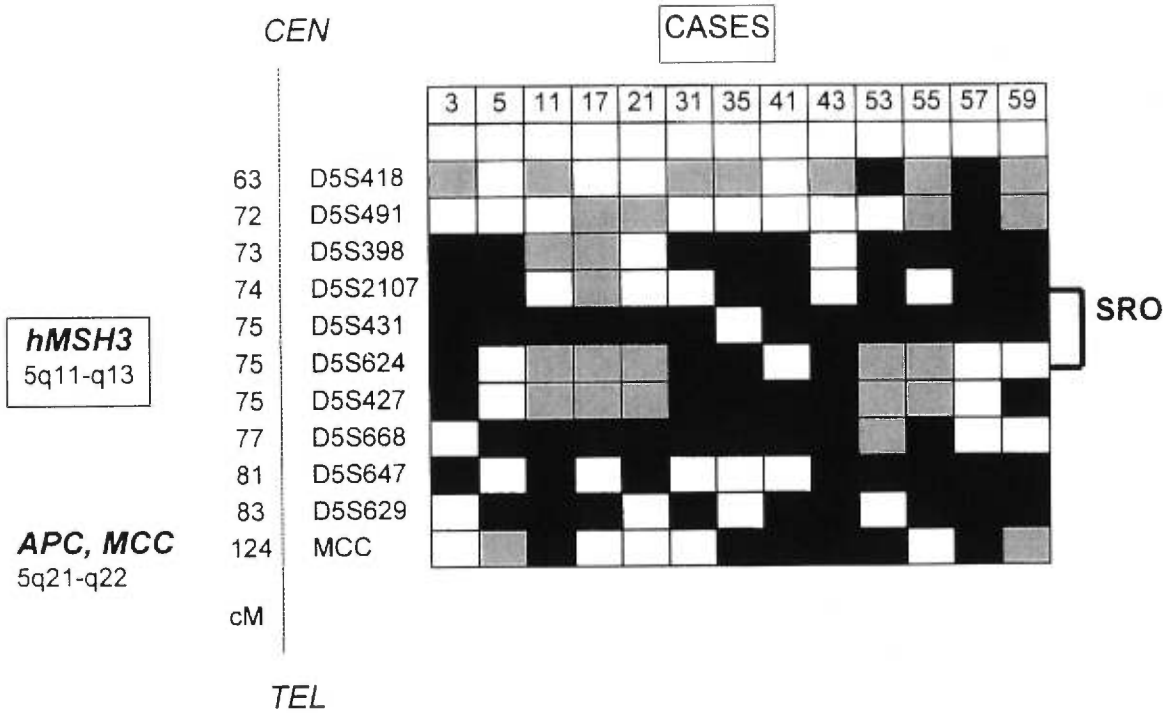


FIGURE 3 – LOH profiles around the 5q11–q13 region and the shortest region of overlapping deletion (SRO).

TABLE III – MICROSATELLITE INSTABILITY IN NON-SMALL CELL LUNG CARCINOMAS

Source	Number of cases	Number of loci tested	Paired typings[2]	Instability			Unstable microsatellites (number of affected patients)
				At 1 locus	At >1 locus	Total	
Peltomäki *et al.* (1993)	90	7	630	2/90 (2%)	None	2/90 (2%)	*D1S216*(2)
Shridhar *et al.* (1994)	38	16	608	5/38 (13%)	7/38 (18%)	12/38 (31%)	*D3S1038*(4), *D3S647*(6), *D3S1076*(3), *D3S966*(7), *Not73*(3), *D3S1289*(5), *D3S659*(6), *D3S1284*(3), *D3S1251*(3), *D3S1215*(2), *D3S1312*(2), *D13S131*(3), *HPRT*(3), *AR*(2), *RTT235*(3), *MEN1*(3)
Fong *et al.* (1995)	108	6	648	6/108 (5%)	1/108 (2%)	7/108 (6%)	*DCC*(3), *D2S123*(2), *MYCL*(3)
Mao *et al.* (1994)	23	9	207	N/A	2/23 (9%)	N/A	*ARA*(1), *D14S50*(1), *AR*(1), *MD*(1), *SAT*(1), *ACTBP2*(1), *FGA*(1), *UT762*(1)
Ryberg *et al.* (1995)	137	5	685	22/137 (16%)	7/137 (5%)	29/137 (21%)	*D3S647*(7), *D3S966*(9), *D3S1286*(10), *D3S1289*(8), *AR*(3)
Wieland *et al.* (1996)	49	7	343	9/49 (18%)	5/49 (10%)	14/49 (29%)	*D3S1289*(5), *D5S491*(6), *D5S637*(7), *D5S626*(1), *D5S644*(1)
Pifarre *et al.* (1997)	64	8	512	20/64 (31%)	22/64 (34%)	42/64 (66%)	*D3S136*(12), *D2S162*(13), *D2S319*(7), *D3S1284*(7), *D3S1289*(3), *D3S1067*(7), *D3S1038*(14), *D3S1611*(10)
This study	31	34	958[3]	None	1/31 (3%)	1/31 (3%)	*D3S1286*(1), *D3S1745*(1), *D3S1561*(1), *D3S1611*(1), *D3S1612*(1), *D3S1298*(1), *D3S3559*(1), *D3S3582*(1), *D3S3647*(1), *D3S1588*(1), *D3S1300*(1), *D3S1312*(1), *D5S2107*(1), *D5S398*(1), *D5S624*(1), *D5S427*(1), *APC*(1), *D2S318*(1), *D2S118*(1), *D2S391*(1), *D2S288*(1), *D7S531*(1), *D7S517*(1)
Total	540	92[1]	4591	64/540 (12%)	45/540 (8%)	109/540 (20%)	228/4591[4] (5%)

[1]Including 75 distinct loci–[2]number of microsatellite typed times number of patients.–[3]Some patients were not analysed with the 34 microsatellites depending on the informativity for LOH and heterozygosity.–[4]Total number of affected loci/total paired typing.

*hMutS*β (Drummond *et al.*, 1995; Palombo *et al.*, 1996), which are then recognized by the heterodimer *hMutL*α composed of *hMLH1/hPMS2*. Therefore, a gene dosage effect affecting the stoichiometry and the activity of heteromolecular mismatch repair complexes may be sufficient to promote cancer. If there is a dosage effect of hMLH1 and hMSH3 on lung cancer progression, it has to

TABLE IV – CHARACTERISTICS OF POLYMORPHISMS DETECTED IN *hMLH1* AND *hMSH3*

Polymorphism	Location	Predicted amino-acid change	Frequency of mutation (%)		
			Allele type	NSCLC patients	Controls
(G/CCpyG/CCAGCG)n	*hMSH3* exon 1	Length polymorphism (polyalanine/proline)	$n = 3$	19	42
			$n = 6$	54	50
			$n = 7$	19	8
			$n = 8$	8	
G → A	*hMSH3* exon 21	$Arg_{940}Gln$	G	88	92
A → G	*hMSH3* exon 23	$Thr_{1036}Ala$	A	62	79
A → G	*hMLH1* exon 8	$Ile_{219}Val$	A	74	67

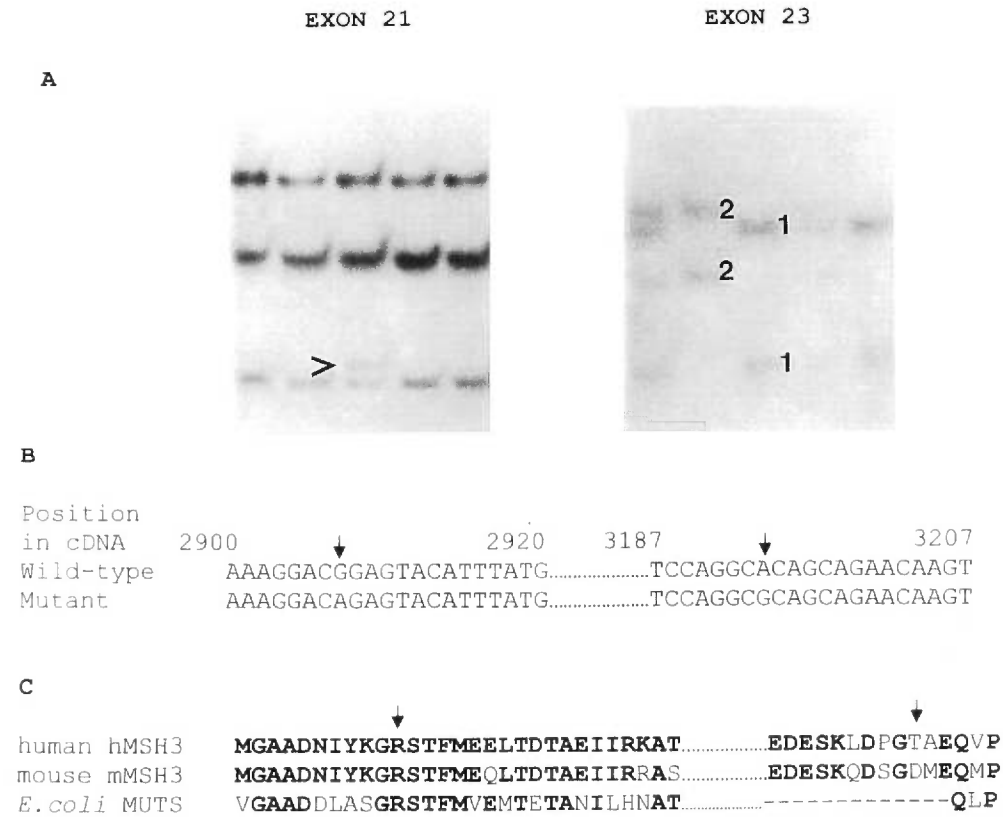


FIGURE 4 – Detection of new *hMSH3* polymorphisms. *a:* SSCP analysis of exon 21 and 23 in five unrelated individuals. Arrowhead indicates an SSCP variant and numbers indicate the single strands corresponding to each allele. *b:* Nucleotide changes corresponding to the SSCP profiles shown in *a* are indicated by arrows. *c:* Partial alignment showing the regions of the MSH3 family of proteins surrounding the sites of the predicted amino-acid polymorphisms (indicated by arrows). Bold type indicates amino-acid identity between at least 2 sequences.

be related to functions other than the replication errors correction, as no RER phenotype was detected in the majority of deleted cases. Mismatch repair proteins are involved in a variety of other vital cellular processes including homologous recombination (de Wind *et al.*, 1995), mediation of the G_2 checkpoint (Hawn *et al.*, 1995), the transcription-coupled nucleotide excision repair involved in the selective removal of DNA damage from the transcribed strands of active genes (Mellon *et al.*, 1996), and the recognition of DNA damage and/or the signaling pathway contributing to the generation of apoptotic cells (Kat *et al.*, 1993). Interestingly, it has been demonstrated that heterozygous as homozygous *hMSH2* mutant mammalian cells have a propensity to accumulate potentially mutagenic oxidative DNA damage, which may promote cancer through defects in genes sensitive to exogenous factors (De Weese *et al.*, 1997; Mellon *et al.*, 1996). Likewise, a heterozygous inactivating mutation in *hMLH1* human-derived cancer cell line was shown to result in significantly reduced transcription-coupled repair (Mellon *et al.*, 1996). These findings raise the possibility that allelic deletion of *hMLH1* and/or *hMSH3* could generate the same effect. A subtle defect in the repair of DNA damage, which is less likely to be lethal to the carrying cells, could have even a more profound impact on tumorigenesis, thus placing individuals at increased cancer risk.

In conclusion, our allelotyping analysis of NSCLC demonstrated that 2 DNA mismatch repair loci, *hMLH1* and *hMSH3*, are frequently affected by LOH at chromosomal regions 3p21 and 5q11–q13, respectively. In both cases, the 1 cM genetic interval associated with the corresponding SRO represents a significant reduction in the previously defined minimally deleted regions. We suggest that *hMLH1* and *hMSH3* loci could promote cancer progression through a dosage effect affecting cellular functions other than the correction of replication errors. Whether or not *hMLH1* and *hMSH3* are real targets of the deletions will have to be

confirmed as the identification of genes with malignancy suppressor activity at 3p21 and 5q11–q13 is extremely important considering the frequency of LOH at these regions in several major forms of cancer. This should provide the opportunity to use these genes and their products to improve the current approaches to the prevention, diagnosis and treatment.

ACKNOWLEDGEMENTS

NB has a fellowship from the Medical Research Council of Canada. DS is a scholar of the Fonds de la recherche en santé du Québec. This work was supported by the Fondation Charles Bruneau and Power Corporation-Fondation de l'Hôpital Sainte-Justine.

REFERENCES

Benachenhou, N., Guiral, S., Gorska-Flipot, I., Laboda D. and Sinnett, D. Frequent loss of heterozygosity of the DNA mismatch-repair loci hMLH1 and hMSH3 in sporadic breast cancer. *Brit. J. Cancer* (1998) (In press).

Boynton, R.F., Blount, P.L., Yin, J., Brown, V.L., Huang, Y., Tong, Y., McDaniel, T., Newkirk, C. and Resau, J.H., Loss of heterozygosity involving the *APC* and *MCC* genetic loci occurs in the majority of human esophageal cancers. *Proc. nat. Acad. Sci. (Wash.).* **89,** 3385–3388 (1992).

Carney, D.N. and DeLeij, L., Lung cancer biology. *Semin. Oncol.*, **15,** 199 (1988).

Chiba, I. and 13 others, Molecular analysis of the short arm of chromosome 3 in small-cell and non-small-cell carcinoma of the lung. *Oncogene*, **5,** 1603–1610 (1990).

Chung, G.T., Sundaresan, V., Hasleton, P., Rudd, R., Taylor, R. and Rabbitts, P.H., Sequential molecular genetic changes in lung cancer development. *Oncogene,* **11,** 2591–2598 (1995).

Daly, M.C., Xiang, R.H., Buchhagen, D., Hensel, C.H., Garcia, D.K., Killary, A.M., Minna, J.D. and Naylor, S.L., A homozygous deletion on chromosome 3 in a small cell lung cancer cell line correlates with a region of tumor suppressor activity. *Oncogene,* **8,** 1721–1729 (1993).

D'Amico, D., Carbone, D.P., Johnson, B.E., Meltzer, S.J. and Minna, J.D., Polymorphic sites within the *MCC* and *APC* loci reveal very frequent loss of heterozygosity in human small cell lung cancer. *Cancer Res.*, **52,** 1996–1999 (1992).

De Weese, T.L., Bucci, J.M., Larrier, N.A., Cutler, R.G., Te Riele, H. and Nelson, W.G., Tolerance of oxidative DNA damage results from disruption of *Msh2* alleles: implications for carcinogenesis. *Proc. Amer. Assoc. Cancer Res.*, **38,** LB-3 (abstract) (1997).

De Wind, N., Dekker, M., Berns, A., Radman, M. and Te Riele, H., Inactivation of the mouse *Msh2* gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell*, **82,** 321–330 (1995).

Drummond, J.T., Li, G.M., Longley, M.J. and Modrich, P., Isolation of an *hMSH2*-p160 heterodimer that restores DNA mismatch repair to tumor cells. *Science,* **268,** 1909–1912 (1995).

Fong, K.M., Zimmerman, P.V. and Smith, P.J., Microsatellite instability and other molecular abnormality in non-small cell lung cancer. *Cancer Res.*, **55,** 28–30 (1995).

Gazdar, A.F., Bader, S., Hung, J., Kishimoto, Y., Sekido, Y., Sugio, K., Virmani, A., Fleming, J., Carbone, D.P. and Minna, J.D., Molecular genetic changes found in human lung cancer and its precursor lesions. *Cold Spring Harbor Symp. quant. Biol.*, **59,** 565–572 (1994).

Han, H.J., Maruyama, M., Baba, S., Park, J.G. and Nakamura,Y., Genomic structure of human mismatch repair gene, *hMLH1*, and its mutation analysis in patients with hereditary non-polyposis colorectal cancer (HNPCC). *Hum. mol. Genet.*, **4,** 237–242 (1995).

Hawn, M.T., Umar, A., Carethers, J.M., Marra, G., Kunkel, T.A., Boland, C.R. AND Koi, M., Evidence for a connection between the mismatch repair system and the G_2 cell cycle checkpoint. *Cancer Res.*, **55,** 3721–3725 (1995).

Hemminki, A., Peltomäki, P., Mecklin, J.P., Jarvinen, H., Salovaara, R., Nystrom-lahti, M., De La Chapelle, A. and Aaltonen, L.A., Loss of the wild-type *MLH1* gene is a feature of hereditary nonpolyposis colorectal cancer. *Nature (Genet.)*, **8,** 405–410 (1994).

Kane, M.F., Loda, M., Gaida, G.M., Lipman, J., Mishra, R., Goldman, H., Jessup, J.M. and Kolodner, R., Methylation of the *hMLH1* promoter correlates with lack of expression of *hMLH1* in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res.*, **57,** 808–811 (1997).

Kat A., Thilly, W.G., Fang, W.H., Longley, M.J., Li, G.M. and Modrich, P., An alkylation-tolerant, mutator human cell line is deficient in strand-specific mismatch repair. *Proc. Nat. Acad. Sci. (Wash.),* **90,** 6424–6428 (1993).

Kok, K., Naylor, S.L. and Buys, C.H., Deletions of the short arm of chromosome 3 in solid tumors and the search for suppressor genes. *Adv. Cancer Res.*, **71,** 27–92 (1997).

Kok K., Osinga, J., Carritt, B., Davis, M.B., Van Der Hout, A.H., Van Der Veen, A.Y., Landsvater, R.M., de Leij, L.F., Berendsen, H.H., Postmus, P.E., Poppema, S. and Buys C.H.C.M., Deletion of a DNA sequence at the chromosomal region 3p21 in all major types of lung cancer. *Nature (Lond.)*, **330,** 578–581 (1987).

Liu, B., Nicolaides, N.C., Markowitz, S., Willson, K.V., Parsons, R.E., Jen, J., Papadopolous, N., Peltomäki, P., de la Chapelle, A., Hamilton, S.R., Kinzler, K.W. and Vogelstein, B., Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. *Nature (Genet.),* **9,** 48–55 (1995).

Mao, L., Lee, D.J., Tockman, M.S., Erozan, Y.S., Askin, F. and Sidransky, D., Microsatellite alterations as clonal markers for the detection of human cancer. *Proc. Nat. Acad. Sci. (Wash.),* **91,** 9871–9875 (1994).

Medeiros, A.C., Nagai, M.A., Neto, M.M. and Brentani, R.R., Loss of heterozygosity affecting the *APC* and *MCC* genetic loci in patients with primary breast carcinomas. *Cancer Epidemiol. Biomarkers Prev.*, **3,** 331–333 (1994).

Mellon, I., Rajpal, D.K., Koi, M., Boland, C.R. and Champe, N., Transcription-coupled deficiency and mutations in mismatch repair genes. *Science*, **272,** 557–560 (1996).

Merlo, A., Mabry, M., Gabrielson, E., Vollmer, R., Baylin, S.B. and Sidransky, D., Frequent microsatellite instability in primary small cell lung cancer. *Cancer Res.*, **54,** 2098–2101 (1994).

Nakajima, E., Orimo, H., Ikejima, M. and Shimada, T., Nine-bp repeat polymorphism in exon 1 of the *hMSH3* gene. *Jpn. J. hum. Genet.*, **40,** 343–345 (1995).

Naylor, S.L., Johnson, B.E., Minna, J.D. and Sakaguchi, A.Y., Loss of heterozygosity of chromosome 3p markers in small-cell lung cancer. *Nature (Lond.),* **329,** 451–454 (1987).

Palombo, F., Iaccarino, I., Nakajima, E., Ikejima, M., Shimada, T. and Jiricny, J., *hMutSbeta*, a heterodimer of *hMSH2* and *hMSH3*, binds to insertion/deletion loops in DNA. *Curr. Biol.* **6,** 1181–1184 (1996).

Papadopoulos, N. , Nicolaides, N.C., Liu, B., Parsons, R., Lengauer, C., Palombo, F., D'Arrigo, A., Markowitz, S., Willson, J.K., Kinzler, K.W., Jiricny, J. and Vogelstein, B., Mutations of *GTBP* in genetically unstable cells. *Science,* **268,** 1915–1917 (1995).

Peltomäki, P. and 12 others, Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. *Cancer Res.*, **53,** 5853–5855 (1993).

Peltomäki, P. and de La Chapelle, A., Mutations predisposing to hereditary nonpolyposis colorectal cancer. *Adv. Cancer Res.*, **71,** 93–119 (1997).

Petersen, I., Langreck, H., Wolf, G., Schwendel, A., Psille, R., Vogt, P., Reichel, M.B., Ried, T. and Dietel, M., Small-cell lung cancer is characterized by a high incidence of deletions on chromosomes 3p, 4q, 5q, 10q, 13q and 17p. *Brit. J. Cancer,* **75,** 79–86 (1997).

Pifarre, A., Rosell, R., Monzo, M., De Anta, J.M., Moreno, I., Sanchez, J.J., Ariza, A., Mate, J.L., Martinez, E. and Sanchez, M., Prognostic value of replication errors on chromosomes 2p and 3p in non-small cell lung cancer. *Brit. J. Cancer,* **75,** 184–189 (1997).

Rabbitts, P., Koga, H., Takahashi, R., Figlin, R.A., Holmes, E.C., Piantadosi, S., Cordon-Cardo, C. and Slamon, D.J., Frequency and extent of allelic loss in the short arm of chromosome 3 in non-small cell lung cancer. *Genes Chromosomes Cancer,* **1,** 95–105 (1989).

Roche, J., Boldog, F., Robinson, M., Robinson, L., Varella-Garcia, M., Swanton, M., Waggoner, B., Fishel, R., Franklin, W., Gemmill, R. and Drabkin, H., Distinct 3p21.3 deletions in lung cancer and identification of a new human semaphorin. *Oncogene*, **12,** 1289–1297 (1996).

Ryberg, D., Lindstedt, B.A., Zienolddiny, S. and Haugen, A., A hereditary genetic marker closely associated with microsatellite instability in lung cancer. *Cancer Res.*, **55,** 3996–3999 (1995).

Shridhar, V., Siegfried, J., Hunt, J., Del Mar Alonso, M. and Smith,

D.I., Genetic instability of microsatellite sequences in many non-small cell lung carcinomas. *Cancer Res.*, **54,** 2084–2087 (1994).

Solomon, E., Voss, R., Hall, V., Bodmer, W.F., Jass, jr., Jeffreys, A.J., Lucibello, F.C., Patel, I. and Rider, S.H., Chromosome 5 allele loss in human colorectal carcinomas. *Nature (Lond.),* **328,** 616–619 (1987).

Sozzi, G. and 13 others, The *FHIT* gene 3p14.2 is abnormal in lung cancer. *Cell,* **85,** 17–26 (1996).

Speicher, M.R. Microsatellite instability in human cancer. *Oncol. Res.*, **7,** 267–275 (1995).

Takahashi, T., Nau, N.M., Chiba, I., Birrer, M.J., Rosenberg, R.K., Vinocour, M., Levitt, M., Pass, H., Gazdar, A.F. and Minna, J.D., *p53*: a frequent target for genetic abnormalities in lung cancer. *Science,* **246,** 491–494 (1989).

Tavassoli, M., Steingrimsdottir, H., Pierce, E., Jiang, X., Alagoz, M., Farzaneh, F. and Campbell, I.G., Loss of heterozygosity on chromosome 5q in ovarian cancer is frequently accompanied by *TP53* mutation and identifies a tumor suppressor gene locus at 5q13.1–21. *Brit. J. Cancer,* **74,** 115–119 (1996).

Tomlinson, I.P., Ilyas, M. and Bodmer, W.F., Allele loss occurs frequently at *hMLH1*, but rarely at *hMSH2*, in sporadic colorectal cancers with microsatellite instability. *Brit. J. Cancer*, **74,** 1514–1517 (1996).

Watanabe, A., Ikejima, M., Suzuki, N., Shimada, T., Genomic organization and expression of the human *MSH3* gene. *Genomics,* **31,** 311–318 (1996).

Wei, Q., Cheng, L., Hog, W.K., and Spitz, M.R., Reduced DNA repair capacity in lung cancer patients. *Cancer Res.* **56,** 4103–4107 (1995).

Wieland, I., Ammermuller, T., Bohm, M., Totzeck, B. and Rajewsky, M.F., Microsatellite instability and loss of heterozygosity at the *hMLH1* locus on chromosome 3p21 occur in a subset of non-small cell lung carcinomas. *Oncol. Res.*, **8,** 1–5 (1996).

Allelic Losses and DNA Methylation at DNA Mismatch Repair Loci in Sporadic Colorectal Cancer

Benachenhou, N.[1], Guiral, S.[1], Gorska-Flipot, I.[2], Michalski, R.[3], Labuda, D.[1,4] and Sinnett, D.[1,4]

[1]Division of Hemato-Oncology, Charles Bruneau Cancer Center, Research Center, Sainte-Justine Hospital; [2]Division of Pathology, Montreal Hôtel-Dieu Hospital; [3]Pathology Department, Victoria Hospital, Prince Albert; [4]Department of Pediatrics, University of Montreal.

NB and SG contributed equally to this work

Corresponding author: Daniel Sinnett, Ph.D., Charles Bruneau Cancer Center, Research Center, Sainte-Justine Hospital, 3175 Côte Ste-Catherine, Montréal (Québec) H3T 1C5, Canada Tel: (514) 345-4931 ext. 2990, Fax: (514) 345-4731

E-mail: sinnettd@ere.umontreal.ca

ABSTRACT

Normal and tumor DNA samples of 35 patients with sporadic colorectal carcinoma were analyzed for microsatellite alterations at 12 markers linked to mismatch repair loci: *hMLH1, hMSH2, hMSH3, hMSH6, hPMS1* and *hPMS2*. Remarkably, no correlation was observed between the replication error phenotype (RER^+) and allelic losses at these loci. Hemizygous deletions, seen in 6 out of the 35 (17%) informative cases at *hMLH1*, 4/27 (15%) at *hMSH2/hMSH6* and 6/34 (18%) at *hMSH3*, were rarely found in RER+ tumors. Since mismatch repair protein components act in molecular complexes of defined stoichiometry we propose that hemizygous deletion of the corresponding loci may be involved in colorectal tumorigenesis through defects in cellular functions other than replication error correction. The analysis of the methylation status of the promoter region of *hMLH1* revealed that methylation might be an important mechanism of this locus inactivation in RER+ sporadic colorectal cancer.

INTRODUCTION

Colorectal cancer is one of the most common forms of cancer in the western world. Its evolution is believed to involve multiple molecular steps through the accumulation of genetic alterations in oncogenes and tumor suppressor genes [1]. Most of the cases are sporadic, although dominant inheritance was suggested in 15% of the patients [2,3]. In hereditary non-polyposis colorectal cancer (HNPCC), the genetic predisposition was shown to be associated with germline mutations in the mismatch repair genes, *hMSH2* [4,5], *hMLH1* [6,7], *hMSH6* [8], *hPMS1* and *hPMS2* [9]. As in tumor suppressor genes, the inactivation of mismatch repair genes seems to occur in two steps: the first mutation, either somatic (sporadic cancer) or germinal (hereditary cancer), is followed by another somatic event. The latter can involve loss of heterozygosity (LOH) as already reported for *hMLH1* in HNPCC and in sporadic cases [10-12].

The absence of mismatch repair leads to deficiency in the correction of replication errors (RER), readily detected as microsatellite instability (RER^+ phenotype) in tumor cells. The RER+ phenotype, characteristic of 92% of HNPCC [13], was observed in 12-28% of sporadic colorectal cancers [14-17]. However, only a subset of the RER^+ sporadic cases were seen associated with mutations in either *hMSH2*, *hMLH1*, *hPMS1* or *hPMS2* [18-20], suggesting a contribution of mutations in other loci [21]. Alternative mechanisms of gene inactivation such as promoter methylation [22] or differential splicing [23] should also be considered.

In this study, aiming sporadic colorectal cancer, we used di- and tetranucleotide markers to simultaneously analyze microsatellite instability and LOH at six mismatch repair loci. While we find no evidence of hemizygous loss of these genes in RER+ tumors,

alternative mechanism such as hypermethylation appears to play an important role in *hMLH1* inactivation.

MATERIALS AND METHODS

Paired normal and tumor samples were obtained from 35 colorectal cancer patients who underwent surgery at the Hôtel-Dieu Hospital in Montreal (n = 29) and the Victoria Hospital in Prince-Albert (n = 6). This is an unselected group of apparently sporadic cases with limited clinical information. DNA was isolated by a standard procedure using digestion with proteinase K and phenol/chloroform extractions.

Matched pairs of normal/tumor DNAs were allelotyped as described in [24] at the following loci linked to *hMLH1* (3p21): D3S1745, D3S1561, D3S1611, D3S1298; to *h*MSH3 (5q11-q13): D5S398, D5S491; to *hMSH2/hMSH6* (2p16): D2S391, D2S288; to *hPMS1* (2q32): D2S318, D2S118, and to *hPMS2* (7p22): D7S531, D7S517. The chromosomal assignment of the genes and microsatellites was performed by integrating genetic, radiation hybrid and STS/YAC data from several sources [25-27]. The change in size of one or both microsatellite alleles in tumor cells was considered as instability, whereas the loss or significant reduction in intensity of one allele in tumor samples compared to the paired normal DNA revealed LOH. Only the informative (heterozygous) loci were included in the estimation of LOH frequency.

The methylation status of the *hMLH1* promoter was tested using a modification of Kane *et al.* assay [22]. Genomic DNA from the patients (10ng), containing an internal control DNA (0.1 pg of a 3.3 kb plasmid construct with 0.3 kb human insert), were

digested for 16 hours using either 12 U of *Hpa*II or 32 U of *Msp*I. The cleavage at the *hMLH1* promoter region was examined by PCR as described [22].

RESULTS

The allelotyping revealed LOH in 15 out of the 35 (43%) tumors analyzed, in at least one of the 12 microsatellite loci tested (Fig. 1, Table 1). Ten tumors (29%) displayed instability (Fig. 2) in at least one of these markers (Table 2), but only 4 (11.4%) unstable at two or more loci were classified RER^+. Interestingly, in these four RER^+ tumors, 7 or more of the 12 microsatellites tested were unstable (Table 2), making the separation between RER^+ and RER^- tumors very distinct.

Six of the patients (17%) exhibited LOH at the *hMLH1* region (Table 1) including the intragenic marker D3S1611 [7]. None of the tumors with LOH at *hMLH1* displayed instability at more than one microsatellite (Tables 2 and 3), suggesting no correlation between the RER^+ phenotype and LOH. Furthermore, the SSCP analysis (not shown) of tumors with LOH failed to detect any mutation in 19 exons of the remaining *hMLH1* allele.

The markers linked to *hMSH2/hMSH6* and *hMSH3* were hemizygously deleted in tumor cells in 4 out of the 27 cases and in 6 out of the 34 cases, respectively (Table 1). Patient 67 was deleted at both regions. In one RER^+ tumor (patient 29), LOH at the *hMSH2/hMSH6* region, seen only at one microsatellite (Table 2), could be a false positive since here the allelic instability could produce a pattern of an allelic loss. None of the other patients with LOH at either *hMSH2/hMSH6* or *hMSH3* had RER^+ tumors (Tables 2

Figure 1. LOH analysis of sporadic colorectal patients using microsatellites markers. Representative allelotypes are shown for patients 23, 25 and 29 with D3S1611, D5S398 and D5S42, respectively. In some cases the deleted allele (indicated by arrow heads) is not entirely absent because normal cells contaminated the tumor sample. N, normal and T, tumoral.

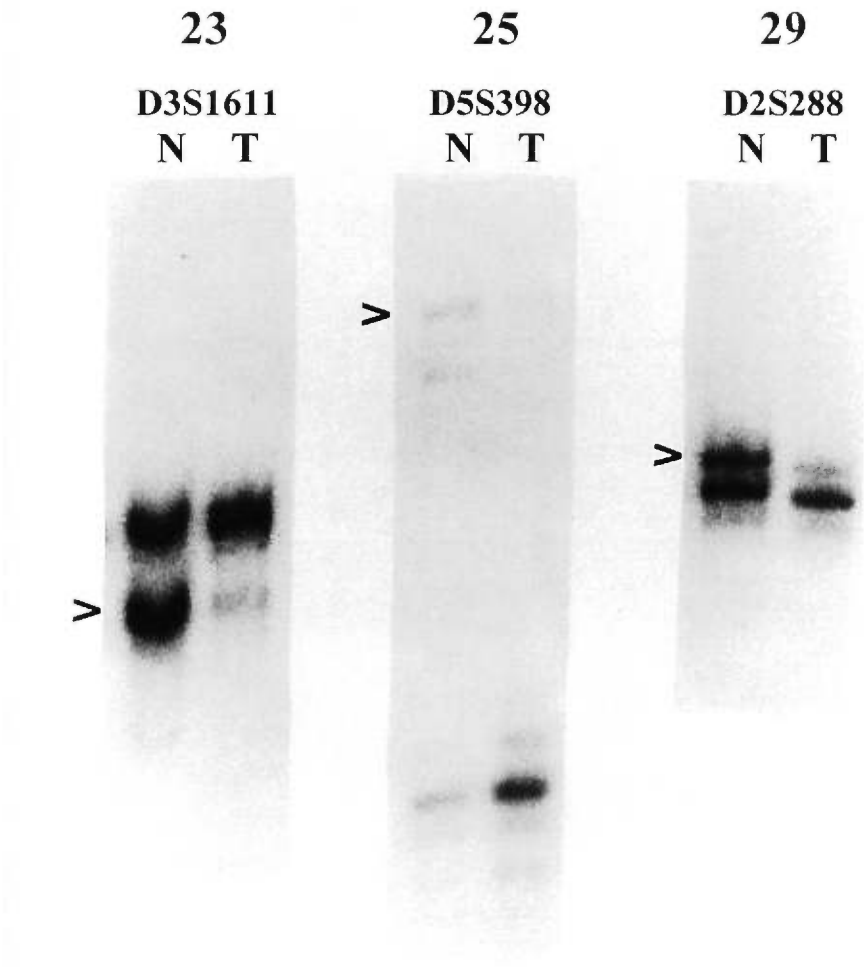


Table 1. LOH results for the 6 analyzed mismatch-repair loci

	MISMATCH-REPAIR LOCUS				
Cases	***hMLH1 (3p21)***	***hMSH2 hMSH6 (2p16)***	***hMSH3 (5q11-q13)***	***hPMS1 (2q32)***	***hPMS2 (7p22)***
1	**LOH**	**LOH**	h	h	h
3	**LOH**	h	h	ni	h
5	h	h	h	h	h
7	h	h	h	h	h
9	h	h	h	h	h
11	h	ni	h	h	h
13*	h	h	h	h	h
15	h	h	h	h	h
17	h	h	h	h	h
19	h	ni	h	h	h
21*	h	h	h	h	**LOH**
23	**LOH**	ni	ni	h	h
25	h	h	**LOH**	h	h
27	**LOH**	ni	h	h	h
29*	h	**LOH**	h	h	h
31	h	h	h	ni	h
33	h	h	h	h	h
35	h	h	h	h	h
37	h	h	**LOH**	h	h
39	h	ni	h	h	h
41*	h	h	h	h	h
43	h	h	h	h	h
45	h	h	h	h	h
47	h	h	h	h	h
49	h	h	h	h	h
51	h	h	h	h	h
53	h	h	**LOH**	h	h
55	**LOH**	h	h	h	h
57	**LOH**	ni	h	h	h
59	h	h	**LOH**	h	h
61	h	h	h	h	h
63	h	ni	**LOH**	h	h
65	h	**LOH**	h	h	h
67	h	**LOH**	**LOH**	h	h
69	h	ni	h	h	h
LOH (%)	**6/35 (17.1%)**	**4/27 (14.8%)**	**6/34 (17.6%)**	**0/33 (0%)**	**1/35 (2.8%)**

LOH: Loss of heterozygosity; h: Heterozygote; ni: Non informative

*RER$^+$ Tumors (see Table 2). *hMSH2* and *hMSH6* are located within 1Mb interval [18].

Figure 2. Detection of microsatellite instability in sporadic colorectal cancer cells. Patient numbers are depicted at the top. Microsatellite markers are shown above each matched normal tumoral DNA pairs. In each case, novel alleles (indicated by filled dots) that were not present in the normal controls (lanes N) are observed in tumors (lanes T).

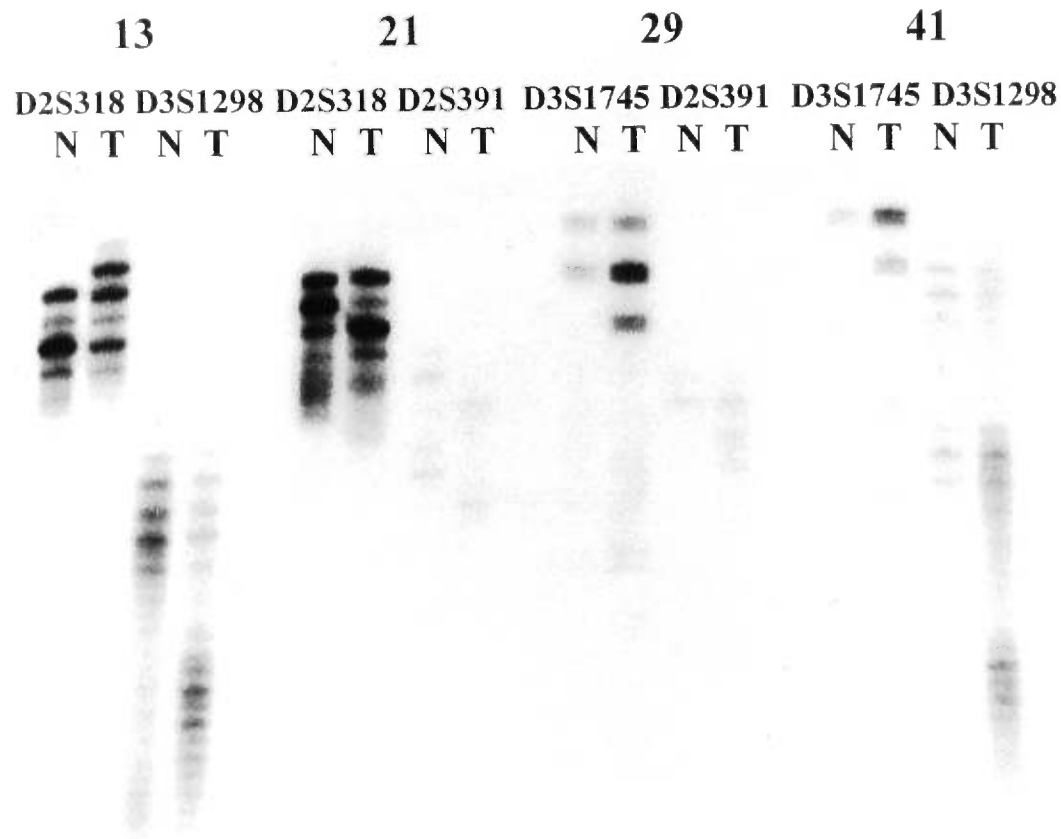


Table 2. Microsatellite instability status for the unstable tumors.

Patients	D3S1745	D3S1561	D3S1611	D3S1298	D2S288	D2S391	D2S118	D2S318	D5S398	D5S491	D7S517	D7S531
11	-	-	-	-	-	-	+	-	-	-	-	-
13[2]	+	+	-	+	+	-	+	+	-	+	-	+
15	-	-	-	-	-	-	+	-	-	-	-	-
21[2]	-	+	-	-	-	+	+	+	+	+	+	-
25	-	-	-	-	-	+	-	-	-	-	-	-
27	-	-	-	-	-	-	-	-	+	-	-	-
29[2]	+	-	+	+	-	+	+	+	-	+	+	+
35	-	+	-	-	-	-	-	-	-	-	-	-
41[2]	+	+	+	+	-	+	+	+	-	+	+	+
59	-	-	-	-	-	-	-	+	-	-	-	-

-: no microsatellite instability detected; + : Microsatellite instability.

[1] The 25 others tumors did not show instability at any of the 12 analyzed microsatellite markers.

[2] Tumors considered as RER^{+}.

Table 3. Relation between the RER^+ phenotype and LOH at mismatch-repair loci

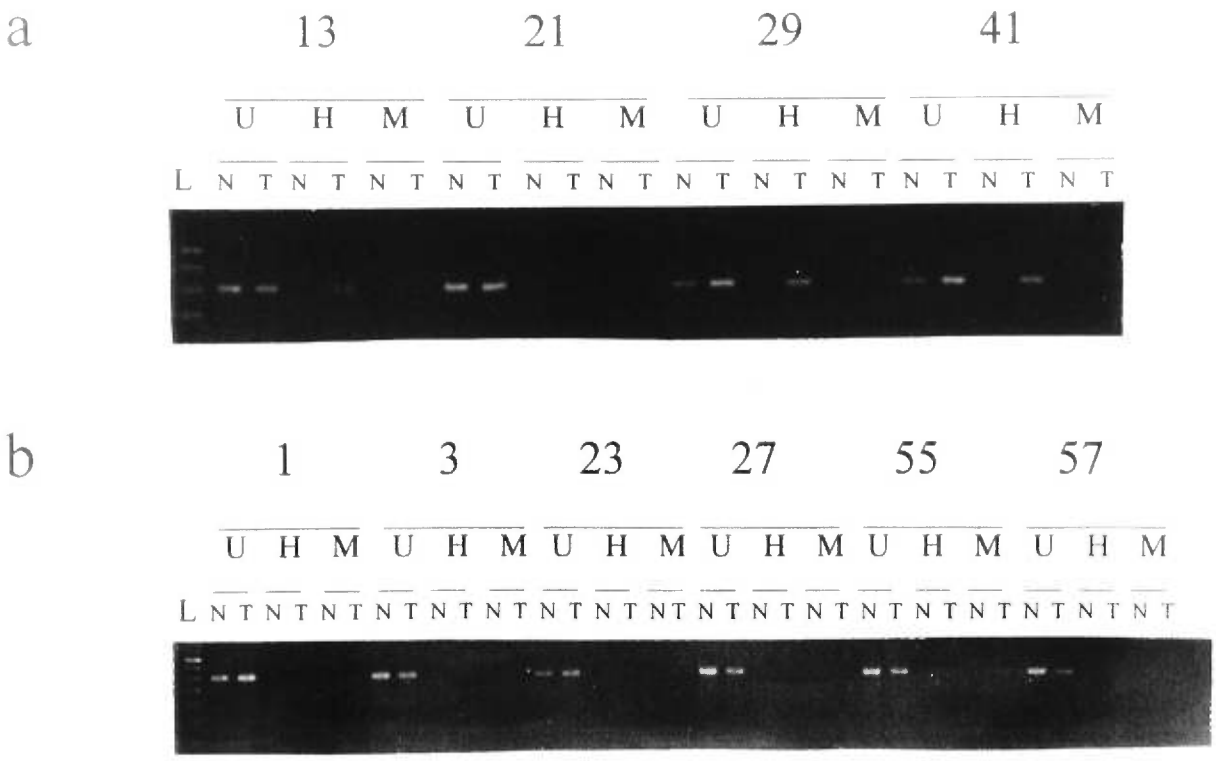
		LOH AT MISMATCH REPAIR LOCI				
		hMLH1	***hMSH2/ hMSH6***	***hMSH3***	***hPMS1***	***hPMS2***
RER status	**RER^- N=31**	6/31 **(19%)**	3/23 **(13%)**	6/30 **(20%)**	0/29	0/31 IC
	RER^+ N=4	0/4	1/4 (25%)	0/4	0/4	1/4 IC (25%)

IC; informative cases

and 3). Few allelic losses at *hPMS2* and *hPMS1* presumably reflect the background level of LOHs in cancer cells rather than a specific effect [28]. One of the RER^+ tumors (case 21) was associated with LOH at *hPMS2* (Tables 2 and 3). Taken together, our results indicate the absence of correlation (p=1, Fisher's test) between LOH at *hMLH1*, *hMSH2/hMSH6* and *hMSH3* and the RER^+ phenotype observed in this group of sporadic colorectal cancer patients (Table 3).

The absence of mutations detectable by SSCP among *hMLH1* exons in RER^+ tumors, prompted us to investigate the methylation status of this gene promoter. The promoter segment (positions –650 to –67), containing four *Hpa*II/*Msp*I sites, was examined for CpG methylation in normal and tumor DNA. Three RER^+ tumor samples (cases 13, 29 and 41) were resistant to *Hpa*II digestion and sensitive to *Msp*I digestion, whereas the corresponding normal DNAs were sensitive to both *Hpa*II and *Msp*I digestions (Fig. 3a). This contrasts with 11 tumor DNAs including samples from the hemizygously deleted *hMLH1* patients (Fig. 3b) and 5 other RER^- tumors (data not shown) with instability at only one microsatellite (Table 2). These were sensitive to digestion with both restriction enzymes indicating no methylation. It appears that the promoter methylation could be a preponderant mechanism of *hMLH1* inactivation in sporadic RER^+ colorectal cancer.

Figure 3. Analysis of sporadic colorectal RER$^+$ and RER$^-$ tumors for methylation of the *hMLH1* promoter region. **a**. Amplification of the *hMLH1* promoter region from the RER$^+$ tumors (T) or the matched normal (N) control, before and after digestion with the indicated restriction endonucleases. **b**. As in a, except that RER$^-$ tumors were analyzed. Patient numbers are shown above each matched normal-tumoral pair. U: undigested; H: digested with *hpa*II; M: digested with *Msp*I.



DISCUSSION

Thirty-five sporadic colorectal carcinomas were investigated for microsatellite alterations in order to determine the relationship between LOH at mismatch repair loci and the RER^+ phenotype. Allelic losses were found at *hMSH2/hMSH6*, *hMLH1*, and at *hMSH3*. We believe this is the first report of LOH at *hMSH3* in colorectal carcinoma. LOH in the *hMSH3*- region was observed earlier in lung and breast carcinomas [29, 30], suggesting its involvement in tumorigenesis. A relatively high level of allelic losses (16%) was also observed at *hMSH2/hMSH6* while only a slight excess (6-9%) of hemizygous deletions in that region over the background level was reported earlier [10, 12]. This difference can be related to the sets of markers assayed. For instance, *D2S391* and *D2S288* which are located at zero recombination fraction (0cM) from *hMSH2* [5] revealed LOH efficiently [10], whereas *D2S123* (located ~3cM from *hMSH2*) was rarely affected by LOH [10,12,14]. The incidence of deletion at *hMLH1* (17%) is similar to that reported in sporadic and hereditary colorectal cancers [10-12]. This except lung and breast cancer where this region was deleted in almost half of cases [29, 30], making it one of the most frequent genetic rearrangements in these neoplasias.

The RER^+ phenotype was observed in 11% of tumors which is consistent with earlier reports [14-17]. However, none of the 4 RER^+ tumors was deleted at *hMLH1* or *hMSH3*. In *hMSH2/hMSH6*, the evidence is weak with LOH in a single patient at only one of the linked markers. The absence of correlation between RER^+ and LOH at mismatch repair genes (Table 3) corroborates our observations with lung and breast cancers [29, 30]. This is in contrast to Tomlison *et al.* [12] reporting LOH at *hMLH1* in 41% sporadic RER^+

colorectal cancers compared to 16% in the RER^- ones. However, in that study the definition of RER^+ phenotype was less stringent than here thus changing substantially their inclusion criteria and the group of patients making these analyses effectively incomparable. This illustrates well the need of uniform inclusion criteria.

The SSCP analysis of *hMLH1* exons in patients hemizygous for this locus failed to reveal any mutation suggesting that the non-deleted copy of this gene remained intact. If the non-deleted *hMLH1*, *hMSH2* or *hMSH3* alleles is active it explains the absence of the RER^+ phenotype. Additional evidence is required to decide whether hemizygous deletions at mismatch repair loci promote cancer progression or whether the observed LOHs indicate only the linkage with as yet unknown tumor suppressor genes. However, it is also possible that a gene dosage effect affecting the stoichiometry and the activity of the heteromolecular mismatch repair complex may be sufficient to promote cancer by impairing functions other than the correction of replication errors. Mismatch repair proteins are known to be involved in a variety of vital cellular functions, such as homologous recombination [31, 32], cell cycle checkpoint control [33], chromosomal synapsis [34], both male and female fertility [34, 35], transcription-coupled nucleotide excision repair [36], the recognition of the DNA damage and/or in the signaling pathway contributing to the generation of apoptotic cells [37]. Noteworthy, DeWeese *et al.* [38] reported that mammalian cells homozygous and hemizygous for disrupted *hMSH2* alleles showed an increase in survival, a decrease in the apoptosis, an increase in the oxidative damage, and an increase in the number of mutations as compared to the normal cells. A heterozygous mutation in *hMSH2* in the human colorectal cancer cell line P8 was shown to reduce transcription-coupled repair [36] suggesting that allelic deletion of *hMLH1* and/or *hMSH3* could have the same effect. In other words, we propose that a subtle defect in the

repair of DNA damage, less likely to be lethal to the affected cells, could have a profound impact on tumorigenesis, placing the carrying individuals at increased cancer risk.

The hypermethylation of the *hMLH1* promoter was correlated with the lack of *hMLH1* expression [22]. Our demonstration that this promoter was methylated in most RER^+ tumors further supports the notion that hypermethylation of *hMLH1* CpG-island might be frequently associated with the inactivation of mismatch repair in RER^+ tumors [22, 39, 40]. This mechanism could explain why not all of the tumors lacking expression of *hMLH1* or *hMSH2* protein had identifiable mutations at these loci [22, 41]. The involvement of an aberrant methylation in carcinogenesis was also reported for growth-regulatory genes (reviewed in 42).

In conclusion, our analysis of sporadic colorectal cancers demonstrated that a majority of RER^+ tumors displayed CpG-methylation of the *hMLH1* promoter region, suggesting this as a possible inactivation mechanism of mismatch repair genes. If this mechanism is indeed operating during cancer development it could have important clinical implications, raising the possibility of reactivation of these silent genes with inhibitors of DNA methylation [42]. Our observation of LOH at mismatch repair loci such as *hMLH1*, *hMSH3* and *hMSH2/hMSH6* in the RER^- cases point to the possibility that a dosage effect affecting mismatch repair genes may promote cancer progression by impairing cellular functions other than the correction of replication errors leading to microsatellite instability.

ACKNOWLEDGEMENTS

N.B. has a fellowship from the MRC of Canada, S.G. has a studentship of the FRSQ, and D.S. is a FRSQ Scholar. This work was supported by the Fondation Charles-Bruneau and the Fondation de l'Hôpital Sainte-Justine/Power Corporation, Inc.

REFERENCES

1. Fearon,E.R. and Vogelstein,B. (1990) A genetic model for colorectal tumorigenesis. *Cell*, **61,** 759-767.

2. Cannon-Albright,L.A., Skolnick,M.H., Bishop,D.T., Lee,R.G. and Burt,R.W. (1988) Common inheritance of susceptibility to colonic adenomatous polyps and associated colorectal cancers. *N. Engl. J. Med.*, **319,** 533-537.

3. Houlston,R.S., Collins,A., Slack,J. and Morton,N.E. (1992) Dominant genes for colorectal cancer are not rare. *Ann. Hum. Genet.*, **56,** 99-103.

4. Fishel,R., Lescoe,M.K., Rao,M.R., Copeland,N.G., Jenkins,N.A., Garber,J., Kane,M. and Kolodner,R. (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell*, **75,** 1027-1038.

5. Leach,F.S., Nicolaides,N.C., Papadopoulos,N., Liu,B., Jen,J., Parsons,R., Peltomaki,P., Sistonen,P., Aaltonen,L.A., Nystrom-Lahti,M., Guan,X.-Y., Zhang,J., Meltzer,P.S., Yu,J.W., Kao,F.-T., Chen,D.J., Cerosaletti,K.M., Fournier,R.E.K., Todd,S., Lewis,T., Leach,R.J., Naylor,S.L., Weissenbach,J., Mecklin,J.-P., Järvinen,H., Petersen,G.M., Hamilton,S.R., Green,J., Jass,J., Watson,P., Lynch,T.T., Trent,J.M., de la Chapelle,A., Kinzler,K.W. and Vogelstein,B. (1993) Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell*, **75,** 1215-1225.

6. Bronner,C.E., Baker,S.M., Morrison,P.T., Warren,G., Smith,L.G., Lescoe,M.K., Kane,M., Earabino,C., Lipford,J., Lindblom,A., Tannergard,P., Bollag,R.J., Godwin,A.R., Ward,D.C., Nordenskjold,M., Fishel,R., Kolodner,R. and Liskay,R.M. (1994) Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature*, **368,** 258-261.

7. Papadopoulos,N., Nicolaides,N.C., Wei,Y.F., Ruben,S.M., Carter,K.C., Rosen,C.A., Haseltine,W.A., Fleischmann,R.D., Fraser,C.M., Adams,M.D., Venter,J.C., Hamilton,S.R., Petersen,G.M., Watson,P., Lynch,H.T., Peltomäki,P., Mecklin,J.-P., de la Chapelle,A., Kinzler,K.W. and Vogelstein,B. (1994) Mutation of a mutL homolog in hereditary colon cancer. *Science*, **263,** 1625-1629.

8. Miyaki, M., Konishi, M., Tanaka, K., Kikuchi-Yanoshita, R., Muraoka, M., Yasuno, M., Igari, T., Koike, M., Chiba, M., Mori, T. (1997) Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. *Nat Genet.*, **17**, 271-272.

9. Nicolaides,N.C., Papadopoulos,N., Liu,B., Wei,Y.F., Carter,K.C., Ruben,S.M., Rosen,C.A., Haseltine,W.A., Fleischmann,R.D., Fraser,C.M., Adams,M.D., Venter,J.C., Dunlop,M.G., Hamilton,S.R., Peterson,G.M., de la Chapelle,A., Vogelstein,B. and Kinzler,K.W. (1994) Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature*, **371,** 75-80.

10. Hemminki,A., Peltomaki,P., Mecklin,J.P., Jarvinen,H., Salovaara,R., Nystrom-Lahti,M., de la Chapelle,A. and Aaltonen,L.A. (1994) Loss of the wild type MLH1 gene is a feature of hereditary nonpolyposis colorectal cancer. *Nat. Genet.*, **8,** 405-410.

11. Jäger,A.C., Bisgaard,M.L., Myrhøj,T., Bernstein,I., Rehfeld,J.F. and Nielsen,F.C. (1997) Reduced frequency of extracolonic cancers in hereditary nonpolyposis colorectal cancer families with monoallelic hMLH1 expression. *Am. J. Hum. Genet.*, **61,** 129-138.

12. Tomlinson,I.P., Ilyas,M. and Bodmer,W.F. (1996) Allele loss occurs frequently at hMLH1, but rarely at hMSH2, in sporadic colorectal cancers with microsatellite instability. *Br. J. Cancer*, **74,** 1514-1517.

13. Liu,B., Parsons,R., Papadopoulos,N., Nicolaides,N.C., Lynch,H.T., Watson,P., Jass,J.R., Dunlop,M., Wyllie,A., Peltomaki,P., de la Chapelle,A., Hamilton,S.R., Vogelstein,B. and Kinzler,K.W. (1996) Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. *Nat. Med.*, **2,** 169-174.

14. Aaltonen,L.A., Peltomaki,P., Leach,F.S., Sistonen,P., Pylkkanen,L., Mecklin,J.P., Jarvinen,H., Powell,S.M., Jen,J., Hamilton,S.R., Peterson,G.M., Kinzler,K.W., Vogelstein,B. and de la Chapelle,A. (1993) Clues to the pathogenesis of familial colorectal cancer. *Science*, **260,** 812-816.

15. Ionov,Y., Peinado,A., Malkhosyan,S., Shibata,D. and Perucho,M. (1993) Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature*, **363,** 558-561.

16. Lothe,R.A., Peltomaki,P., Meling,G.I., Aaltonen,L.A., Nystrom-Lahti,M., Pylkkanen,L., Heimdal,K., Andersen,T.I., Moller,P., Rognum,T.O., Fossa,S.D., Haldorsen,T., Langmark,F., Brogger,A., de la Chapelle,A. and Borresen,A.-L. (1993) Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. *Cancer Res.*, **53,** 5849-5852.

17. Thibodeau,S.N., Bren,G. and Schaid,D. (1993) Microsatellite instability in cancer of the proximal colon. *Science*, **260,** 816-819.

18. Liu,B., Nicolaides,N.C., Markowitz,S., Willson,J.K., Parsons,R.E., Jen,J., Papadopolous,N., Peltomaki,P., de la Chapelle,A., Hamilton,S.R., Kinzler,K.W. and Vogelstein,B. (1995) Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. *Nat. Genet.*, **9,** 48-55.

19. Dunlop,M.G. (1997) Colorectal cancer. *BMJ*, **314,** 1882-1885.

20. Moslein,G., Tester,D.J., Lindor,N.M., Honchel,R., Cunningham,J.M., French,A.J., Halling,K.C., Schwab,M., Goretzki,P. and Thibodeau,S.N. (1996) Microsatellite instability and mutation analysis of hMSH2 and hMLH1 in patients with sporadic, familial and hereditary colorectal cancer. *Hum. Mol. Genet.*, **5,** 1245-1252.

21. Lewis, C.M., Neuhausen, S.L., Daley, D., Black, F.J., Swensen, J., Burt, R.W., Cannon-Albright, L.A. and Skolnick, M.H. (1996) Genetic heterogeneity and unmapped genes for colorectal cancer. *Cancer Res.*, **56**,1382-1388.

22. Kane,M.F., Loda,M., Gaida,G.M., Lipman,J., Mishra,R., Goldman,H., Jessup,J.M. and Kolodner,R. (1997) Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res.*, **57,** 808-811.

23. Charbonnier,F., Martin,C., Scotte,M., Sibert,L., Moreau,V. and Frebourg,T. (1995) Alternative splicing of MLH1 messenger RNA in human normal cells. Cancer Res., **55**, 1839-1841.

24. Baccichet,A., Benachenhou,N., Couture,F., Leclerc,J.-M. and Sinnett,D. (1997) Microsatellite instability in childhood T cell acute lymphoblastic leukemia. *Leukemia,* **11,** 797-802.

25. Gyapay,G., Morissette,J., Vignal,A., Dib,C., Fizames,C., Millasseau,P., Marc,S., Bernardi,G., Lathrop,M. and Weissenbach,J. (1994) The 1993-94 Genethon human genetic linkage map. *Nat. Genet.*, **7,** 246-339.

26. Hudson,T.J., Stein,L.D., Gerety,S.S., Ma,J., Castle,A.B., Silva,J., Slonim,D.K., Baptista,R., Kruglyak,L., Xu,S.-H., Hu,X., Colbert,A.M.E., Rosenberg,C., Reeve-Daly,M.P., Rozen,S., Hui,L., Wu,X., Vestergaard,C., Wilson,K.M., Bae,J.S., Maitra,S., Ganiatsas,S., Evans,C.A., DeAngelis,M.M., Ingalls,K.A., Nahf,R.W., Horton,Jr.,L.T., Anderson,M.O., Collymore,A.J., Ye,W., Kouyoumjian,V., Zemsteva,I.S., Tam,J., Devine,R., Courtney,D.F., Renaud,M.T., Nguyen,H., O'Connor,T.J., Fizames,C., Fauré,S., Gyapay,G., Dib,C., Morissette,J., Orlin,J.B., Birren,B.W., Goodman,N., Weissenbach,J., Hawkins,T.L., Foote,S., Page,D.C. and Lander,E.S. (1995) An STS-based map of the human genome. *Science,* **270,** 1945-1954.

27. Gemmill,R.M., Chumakov,I., Scott,P., Waggoner,B., Rigault,P., Cypser,J., Chen,Q., Weissenbach,J., Gardiner,K., Wang,H., Pekarsky,Y., Le Gall,I., Le Paslier,D, Guillou,S., Li,E., Robinson,L., Hahner,L., Todd,S., Cohen,D. and Drabkin,H.A. (1995) A second-generation YAC contig map of human chromosome 3. *Nature,* **377,** 299-319.

28. Vogelstein,B., Fearon,E.R., Kern,S.E., Hamilton,S.R., Preisinger,A.C., Nakamura,Y., White,R. (1989) Allelotype of colorectal carcinomas. *Science*, **244,** 207-211.

29. Benachenhou,N., Guiral,S., Gorska-Flipot,I., Labuda,D. and Sinnett,D. (1998) High resolution deletion mapping reveals frequent allelic losses at the DNA mismatch repair loci *hMLH1* and *hMSH3* in non-small cell lung cancer. *Int. J. Cancer,* **77**, 173-180.

30. Benachenhou,N., Guiral,S., Gorska-Flipot,I., Labuda,D. and Sinnett,D. (1998) Frequent loss of heterozygosity at the DNA mismatch-repair loci *hMLH1* and *hMSH3* in sporadic breast cancer. *Br. J. Cancer* (in press).

31. de Wind,N., Dekker,M., Berns,A., Radman,M. and te Riele,H. (1995) Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell*, **82,** 321-330.

32. Sugawara,N., Paques,F., Colaiacovo,M. and Haber,J.E. (1997) Role of *Saccharomyces cerevisiae* Msh2 and Msh3 repair proteins in double-strand break-induced recombination. *Proc. Natl. Acad. Sci. USA*, **94,** 9214-9219.

33. Hawn,M.T., Umar,A., Carethers,J.M., Marra,G., Kunkel,T.A., Boland,C.R. and Koi,M. (1995) Evidence for a connection between the mismatch repair system and the G2 cell cycle checkpoint. *Cancer Res.*, **55**, 3721-3725.

34. Baker,S.M., Bronner,C.E., Zhang,L., Plug,A.W., Robatzek,M., Warren,G., Elliott,E.A., Yu,J., Ashley,T., Arnheim,N., Flavell,R.A. and Liskay,R.M. (1995) Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis. *Cell,* **82,** 309-319.

35. Baker,S.M., Plug,A.W., Prolla,T.A., Bronner,C.E., Harris,A.C., Yao,X., Christie,D-M., Monell,C., Arnheim,N., Bradley,A., Ashley,T. and Liskay,R.M. (1996) Involvement of mouse *Mlh1* in DNA mismatch repair and meiotic crossing over. *Nat. Genet.*, **13,** 336-342.

36. Mellon,I., Rajpal,D.K., Koi,M., Boland,C.R. and Champe,G.N. (1996) Transcription-coupled repair deficiency and mutations in human mismatch repair genes. *Science,* **272**, 557-560.

37. Kat,A., Thilly,W.G., Fang,W.H., Longley,M.J., Li,G.M. and Modrich,P. (1993) An alkylation-tolerant, mutator human cell line is deficient in strand-specific mismatch repair. *Proc. Natl. Acad. Sci. USA,* **90**, 6424-6428.

38. De Weese,T.L., Bucci,J.M., Larrier,N.A., Cutler,R.G., te Riele,H. and Nelson,W.G. (1997) Tolerance of oxidative DNA damage results from disruption of Msh2 alleles: implications for carcinogenesis. *Proceedings, 88th Annual Meeting, American Association for Cancer Research*, San Diego, CA, **38**, 540.

39. Christensen,E.R., Cunningham,J.M., Tester,D.J., Roche,P.C., Burgart,L.J. and Thibodeau,S.N. (1998) Hypermethylation of the *hMLH1* promoter in colon cancer with microsatellite instability. *Proceedings, 89th American Association for Cancer Research*, New Orleans, LA, **39**, 60.

40. Esteller,M., Levine,R., Baylin,S.B., Hedrick Ellenson,L. and Herman,J.G. (1998) *hMLH1* promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. *Proceedings, 89th American Association for Cancer Research*, New Orleans, LA, **39**, 538.

41. Thibodeau,S.N., French,A.J., Roche,P.C., Cunningham,J.M., Tester,D.J., Lindor,N.M., Moslein,G., Baker,S.M., Liskay,R.M., Burgart,L.J., Honchel,R. and Halling,K. (1996) Altered expression of hMSH2 and hMLH1 in tumors with microsatellite instability and genetic alterations in mismatch repair genes. *Cancer Res.*, **56,** 4836-4840.

42. Jones,P.A. (1996) DNA methylation errors and cancer. *Cancer Res.*, **56,** 2463-2467.

DISCUSSION

L'altération du système de réparation de bases mésappariées (SRM) est bien documentée dans HNPCC et les cancers sporadiques colorectaux (revue dans Peltomaki et de la Chapelle, 1997 ; Leach *et al.*, 1993 ; Papadopoulos *et al.*, 1994 ; Bronner *et al.*, 1994 ; Liu *et al.*, 1995 ; Tomlinson *et al.*, 1996). Une déficience dans cette voie de réparation de l'ADN mène à l'apparition d'erreurs de réplication à travers le génome qui peuvent être révélées au niveau de marqueurs microsatellites (Fishel et Kolodner, 1995 ; Wooster *et al.*, 1994 ; Aaltonen *et al.*, 1993, Thibodeau *et al.*, 1993). De plus, il a été suggéré qu'une déficience en réparation serait impliquée dans la tumorigenèse du sein et du poumon (Helzlsouer *et al.*, 1996 ; Parshad *et al.*, 1996 ; Yee *et al.*, 1994 ; Karnik *et al.*, 1995 ; Paulson *et al.*, 1996 ; Wei *et al.*, 1995 ; Pifarre *et al.*, 1997 ; Shridhar *et al.*, 1994 ; Wieland *et al.*, 1996). Mon projet avait pour but de vérifier l'implication du SRM dans l'étiologie des cancers sporadiques du sein, du poumon et du côlon.

EXPRESSION D'UN PHÉNOTYPE MUTATEUR DANS LA FORME SPORADIQUE DES CANCERS DU SEIN, DU POUMON ET DU CÔLON

Un phénotype mutateur a été observé chez 4 des 35 (11%) tumeurs colorectales analysées, une fréquence semblable aux autres études portant sur le même type de cancer (Aaltonen *et al.*, 1993 ; Ionov *et al.*, 1992 ; Lothe *et al.*, 1993 ; Thibodeau *et al.*, 1993). Nous avons aussi démontré que l'instabilité de microsatellite est un phénomène rarement observé chez les cancers du sein (0/22) et les cancers du poumon (1/31). Nos résultats sont en accord avec la plupart des études effectuées sur les tumeurs du sein et du poumon. La compilation des données de la littérature sur 362 cancers du sein indique que seulement 2.2% (84/3724)

des cas analysés expriment un phénotype mutateur (Tableau V, pp. 86). Pareillement, en compilant les données de la littérature pour 540 cas de cancer du poumon autres qu'à petites cellules (CPAPC), seulement 5% (228/4591) des cas analysés exprime un phénotype mutateur (Table III , pp. 56). À partir de la compilation présente au Tableau V, nous avons estimé le taux moyen de mutations somatiques de microsatellites à 5×10^{-3} (12/2556). Une valeur très similaire à celle obtenue pour les lymphocytes-T (3×10^{-3}) (Hackman *et al.,* 1995), renforçant notre conclusion qu'une déficience dans la correction d'erreurs commises lors de la réplication ne joue pas ou presque pas de rôle dans le cancer du sein et du poumon.

PERTES D'HÉTÉROZYGOTIE DANS LES CAS DE CANCER DU SEIN ET DU POUMON

De nombreuses études pour les cas de cancer du sein et du poumon non-héréditaire ont démontrées des pertes alléliques pour un grand nombre de bras chromosomiques, suggérant ainsi l'implication de plusieurs gènes suppresseurs de tumeurs (Tableau II, pp. 10 ; Sato *et al.,* 1990 ; Cornelisse *et al.,* 1992 ; Bieche *et al.,* 1995 ; Peterson *et al.,* 1997). Notre allélotypage des gènes SRM a montré que les régions chromosomiques 3p21 et 5q11-q13 sont délétées de façon hémizygote dans 46% et 23% des tumeurs du sein et dans 55% et 42% des tumeurs du poumon, respectivement.

DÉLÉTION DU CHROMOSOME 3p21

La délétion du chromosome 3p est l'un des plus fréquents réarrangements génétiques observés dans les cellules cancéreuses (Kok *et al.,* 1997 ; Pandis *et al.,* 1993). Des PDH

Tableau V. Compilation d'instabilités de microsatellites dans les cancers sporadiques du sein

Source	# of cases	# of loci tested	Paired typing[3]	Instability			Unstable microsatellites (# of affected cases)
				at 1 locus	at > 1 loci	Total	
Han et al. 1993	26	4	104	1/26 (4%)	none	1/26 (4%)	p53
Lothe et al. 1993	84	7	588	none	none	none	none
Wooster et al. 1994	104	12	1248	11/104 (11%)	none	11/104 (11%)	D2S123(1), AR(4), DM1(2), vWFa(2), vWFb(1), DXS981(1)
Yee et al. 1994	20	7	140	1/20 (5%)	3/20 (15%)	4/20 (20%)	15q11(3), D17S807 (3), D18S34(2), AR(2), alu(1)
Karnik et al. 1995	69	9	621	19/69 (28.5%)	1/69 (1.5%)	20/69 (29%)	D11S988(20),D11S1760(1), D13S175(1), D17S807(1)
Paulson et al. 1996	37	11	407	14/37 (38%)	11/37 (30%)	25/37 (68%)	D17S579(1), D17S250(3), D17S855(1), ATA7G11(2), p53(2), GATA8E01(1),GATA11B12(2), GGAA4D07(11), GGAA2E02(15)
This study	22	44[1]	616[5]	none	none	none	none
Total	362	94[2]	3724	46/362 (12.7%)	15/362 (4.1%)	61/362 (16.8%)	84/3724 (2.2%)[4]

[1]Including GGAA4D07 and GGAA2ED2 from Paulsen et al. 1996; [2]including 88 distinct loci; [3] number of microsatellite typed x number of patients; [4] total number of affected loci/total paired typing; [5]Some patients were not analysed with the 44 microsatellites depending of the informativity for LOH and heterozigosity.

au niveau du chromosome 3p ont été rapportées dans 100% des cas de cancer du poumon à petites cellules (CPPC) (revue dans Kok *et al.*, 1987 ; Naylor *et al.*, 1987) et dans plus de 50% des cas de CPAPC (Kok *et al.*, 1987 ; Rabbitts *et al.*, 1989). Chez des lignées cellulaires dérivées de tumeurs du poumon ainsi que chez des tumeurs primaires, la présence de délétions homozygotes a été rapportée pour le chromosome 3p (revue dans Kok *et al.*, 1997 ; Roche *et al.*, 1996). Plus particulièrement, une région du chromosome 3p, soit la région 3p21.3 (SCLC), a été trouvée comme étant délétée de façon homozygote chez des lignées cellulaires de CPPC (Daly *et al.*, 1993). Dans le cancer du sein, des PDH ont été trouvées dans 30-47% des patients dans 2 régions distinctes, soit 3p13-p14 et 3p21-p25 (Chen *et al.*, 1994 ; Matsumoto *et al.*, 1997). De plus, une activité de suppression de tumeurs pour le chromosome 3 au complet ou en partie a été démontrée par plusieurs groupes (revue dans Kok *et al.*, 1997). Ces observations supportent la présence d'un ou plusieurs gènes suppresseurs de tumeurs sur le chromosome 3p.

Le gène *PTPRG* qui code pour une protéine-tyrosine phosphatase (Sorio *et al.*, 1995) et le gène *FHIT* codant pour une diadénosine triphosphate hydrolase (Barnes *et al.*, 1996), sont tous deux localisés à l'intérieur de la région 3p13-p14 et par le fait même sont deux candidats potentiels comme cibles des délétions dans les tumeurs du sein et du poumon (LaForgia *et al.*, 1991 ; Ohta *et al.*, 1996 ; Negrini *et al.*, 1996). Notre analyse d'allélotypage a permis de restreindre la région de délétion du chromosome 3p21 à un intervalle compris entre les marqueurs *D3S1286* et *D3S1298* (Figure 2, pp. 32) pour les cas de cancer du sein et les marqueurs *D3S1561* et *D3S1612* (Figure 2, pp. 55) pour les cas de cancer du poumon, excluant ainsi *FHIT* et *PTPRG* de nos régions minimales communes de délétion. Notre analyse a permis de réduire significativement la taille maximum de la région associée au locus candidat (un intervalle de moins de 1 cM pour le cancer du

poumon). Le seul gène candidat connu et présent dans cette région délétée est *hMLH1*. Est-ce que la délétion hémizygote de ce gène a pour effet de promouvoir la progression du cancer du sein ou la progression du cancer du poumon? Ou bien est-il seulement en liaison avec un gène suppresseur encore inconnu ? Ces questions restent présentement sans réponse, mais nous spéculerons plus loin (pp. 91-92) sur la signification de ces résultats.

DÉLÉTION DU CHROMOSOME 5q11-q13

Des délétions au niveau du chromosome 5q ont été rapportées dans plusieurs tumeurs dont celles affectant le côlon (Solomon *et al.*, 1987 ; Vogelstein *et al.*, 1988 ; Ashton-Rickart *et al.*, 1991), le poumon (Ashton-Rickart *et al.*, 1991 ; D'Amico *et al.*, 1992), le sein (Medeiros *et al.*, 1994 ; Benachenhou *et al.*, 1998b), les ovaires (Tavassoli *et al.*, 1996) et l'oesophage (Boynton *et al.*, 1992). Il existe au moins deux gènes suppresseurs connus dans cette région du génome : *APC* et *MCC*. Certaines délétions chevauchent la région de *APC* et *MCC*, mais les limites des régions minimales de délétions comprises entre les marqueurs *D5S418* et *D5S647* pour les cas de cancer du sein et entre les marqueurs *D5S2107* et *D5S624* pour les cas de cancer du poumon (un intervalle génétique de moins de 1 cM), ont permis d'exclure ces loci dans ~ 50% des cas délétés (Figure 3, pp. 34 et Figure 3, pp. 56). D'ailleurs, la région minimale de délétion se retrouve à une distance génétique de plus de 40 cM de *APC* et *MCC*. Des pertes alléliques dans 33% des cas de cancer utérin au niveau de 5q13.1-q21 ont aussi été rapportées comme étant à l'extérieur de la région comprenant *APC* et supportent nos observations (Tavassoli *et al.*, 1996). Donc, des gènes autres que *APC/MCC* doivent être considérés comme cibles des délétions

observées dans la région 5q11-q13. Le gène *hMSH3* est un bon candidat comme cible de ces délétions mais on ne peut exclure la présence d'autres gènes présentement inconnus.

PERTE D'HÉTÉROZYGOTIE CHEZ LES CAS DE CANCER DU CÔLON

Notre étude de 35 cas de cancer sporadique du côlon nous a permis d'observées des pertes alléliques aux loci d'*hMSH2/hMSH6*, d'*hMLH1*, et d'*hMSH3*. Ceci est la première fois que la présence de PDH pour *hMSH3* est présentée pour des cas de cancer sporadique du côlon. Ayant déjà démontré ci-dessus que la région associée à *hMSH3* est un site fréquent de PDH dans les cas sporadiques de cancer du sein et du poumon, nous suggèrons un rôle général pour ce gène dans ces tumorigenèse.

Un taux de délétions relativement élevé (16%) a été observé pour la région d'*hMSH2/hMSH6*. D'autres études ont rapporté un petit excès (6-9%) de délétions hémizygotes en comparaison au niveau de base de PDH pour cette région (Hemminki *et al.*, 1994; Tomlinson *et al.*, 1996). Ceci peut s'expliqué en partie par une différence au niveau du choix des marqueurs analysés. Par exemple, les marqueurs *D2S391* et *D2S288* qui sont localisés à 0 fraction de recombinaison (0cM) de *hMSH2* (Leach *et al.*, 1993) démontrent plusieurs PDH (notre étude ; Hemminki *et al.*, 1994). Cependant, le marqueur *D2S123* (localisé à ~3cM d'*hMSH2*) est rarement affecté par des PDH (Aaltonen *et al.*, 1993 ; Hemminki *et al.*, 1994 ; Tomlinson *et al.*, 1996).

La fréquence de délétions observées pour *hMLH1* est de 17%. Cette fréquence est similaire aux 23% et 25% obtenus dans les cas de cancer du côlon héréditaire (Hemminki

et al., 1994 ; Jäger *et al.,* 1997) et sporadique (Tomlinson *et al.,* 1996). À noter, le locus *hMLH1* est délété dans presque la moitié des cas de cancer du poumon et du sein analysés ci-dessus (Benachenhou *et al.,* 1998a,b), faisant de cette région l'une des cibles privilégiées de réarrangements dans ces néoplasies.

CORRÉLATION ENTRE LA PRÉSENCE DE PDH ET L'EXPRESSION D'UN PHÉNOTYPE MUTATEUR

Notre analyse a démontré que seulement un cas de cancer du poumon exprime un phénotype mutateur. Cependant, aucune tumeur du sein et du poumon, hémizygote pour les gènes *hMLH1* et *hMSH3,* n'exprime ce phénotype. L'analyse mutationnelle par *SSCP* pour *hMLH1* et *hMSH3* n'a pas révélée de mutations ponctuelles pour aucun cas. L'intégrité de l'allèle non-délété pour *hMLH1* et *hMSH3* expliquerait l'absence de phénotype mutateur. La présence de délétions simultanées d'*hMSH3* et d'*hMLH1* pour plusieurs patients (Table I, pp. 31 et Table II, pp. 54) soulève d'intéressantes questions au sujet de leur relation. Il est connu que les protéines *hMSH2* et *hMSH6* ou bien *hMSH2* et *hMSH3* se lient au mésappariement comme un hétérodimère nommé respectivement *hMutSα* et *hMutSβ* (Drummond *et al.,* 1995 ; Palombo *et al.,* 1996). Ce complexe est par après reconnu par un autre hétérodimère composé d'*hMLH1* et *hPMS2* nommé *hMutLα.* Un effet de dosage de ces gènes affectant la stoechiométrie et l'activité de ces complexes de réparation pourrait être suffisant pour promouvoir le cancer en altérant de façon négative certains processus cellulaires autres que la réparation d'erreurs commises lors de la réplication. En effet, les gènes de réparation de mésappariements de bases de l'ADN

sont impliqués dans une variété de processus cellulaires vitaux dont la recombinaison homologue (deWind *et al.*, 1995), la médiation du G2 *checkpoint* (Hawn *et al.*, 1995), la synapse chromosomique (Baker *et al.*, 1995), la fertilité masculine (Baker *et al.*, 1995), dans le *transcription-coupled nucleotide excision repair* (TCR) où il y a une réparation préférentielle du brin transcrit des gènes actifs (Mellon *et al.*, 1996) et dans la reconnaissance de dommage à l'ADN et/ou dans la signalisation menant à la génération de cellules apoptotiques (Kat *et al.*, 1993 ; Mu *et al.*, 1997). De plus, il a été démontré que des délétions hétérozygotes et homozygotes d'*hMSH2* mènent à l'accumulation de dommages oxidatifs potentiellement mutagéniques au niveau de l'ADN (DeWeese *et al.*, 1997). Pareillement, une mutation hétérozygote inactivante d'*hMLH1* présente dans une lignée cellulaire dérivée d'un cancer humain mène à une déficience dans le processus de réparation préférentielle du brin transcrit de gènes actifs (TCR) (Mellon *et al.*, 1996). Ces résultats soulèvent la possibilité que des délétions hémizygotes d'*hMLH1* et/ou d'*hMSH3* pourraient produire le même effet.

Ces résultats sur les cancers du sein et du poumon sont supportés par l'absence de corrélation entre l'expression d'un phénotype mutateur et de PDH au niveau des loci *hMLH1*, *hMSH2/hMSH6* et *hMSH3* chez les cas de cancers du côlon analysés dans cette étude. Nos résultats contrastent avec ceux de Tomlison *et al.* (1996), qui démontrent des PDH pour *hMLH1* dans 41% des cas de cancer sporadique du côlon ayant un phénotype mutateur comparativement à 16% pour les cas sans phénotype mutateur. Cependant dans cette étude, les critères de sélection des patients ainsi qu'une définition moins stricte d'un phénotype mutateur rendent difficile la comparaison des résultats. L'analyse mutationnelle de *hMLH1* chez les cas hémizygotes de ce gène n'a pas révélé de mutations, suggérant que l'allèle non-délété n'est pas inactivé par des mutations ponctuelles. La présence d'un allèle

résiduel non-muté pourrait expliquer l'absence d'un phénotype mutateur. Est-ce que le statut d'hétérozygotie pour une mutation dans un gène SRM joue un rôle dans la tumorigenèse du côlon ? Il est possible, comme décrit ci-dessus, qu'un effet de dosage puisse affecter d'autres processus cellulaires que la correction d'erreurs de réplication.

L'instabilité génétique est une caractéristique nécessaire de la tumorigenèse (Loeb, 1991; Hartwell, 1992 ; Nowell, 1976). Chez le cancer du côlon, cette instabilité s'exprime de deux façons : une portion des tumeurs (~15%) sont déficientes au niveau des gènes SRM, alors que le reste des tumeurs sont aneuploïdes due à une instabilité chromosomique (Lengauer *et al.,* 1997 ; Hartwell *et al.,* 1992). Il semble que la cellule tumorale nécessite la présence d'une forme d'instabilité quelque soit la nature de celle-ci. De façon générale, il existe une relation inverse entre le taux de réarrangements des chromosomes et les instabilités de microsatellites (Remvikos *et al.,* 1995).

À la lumière de ces résultats, nous proposons qu'un changement subtil au niveau de la réparation des dommages à l'ADN, moins létal pour la cellule, pourrait avoir un effet à long terme encore plus marquant sur la tumorigenèse, plaçant ainsi les individus devant un risque plus élevé de développer un cancer.

AUTRES MÉCANISMES D'INACTIVATIONS

Outre les pertes alléliques, il existe d'autres mécanismes qui peuvent mener à l'inactivation d'un gène. Les mutations ponctuelles, la méthylation du promoteur, de petites délétions ainsi que des réarrangements de gènes en sont tous des exemples. Nous avons vérifiée

l'implication de certains de ces mécanismes au niveau des gènes SRM chez nos tumeurs. L'analyse mutationnelle par *SSCP* de la région codante du gène *hMLH1* dans les tumeurs hémizygotes pour ce loci ainsi que celles exprimant un phénotype mutateur n'a pas révélé la présence de mutations inactivantes. L'analyse mutationnelle du gène *hMSH3* a permis d'identifier 3 variants incluant un polymorphisme connu pour l'exon 1 d'*hMSH3* (Table IV, pp. 57 ; Nakajima *et al.,* 1995). L'implication des deux autres mutations trouvées pour *hMSH3,* dont le résultat est un changement en acide aminé ($Arg_{940}Gln$ et $Thr_{1036}Ala$), reste à être déterminée. Cependant, la présence de ses variants dans nos échantillons contrôles semble indiquer que ces changements sont des polymorphismes plutôt que des changements pathogènes. En d'autres mots, aucune mutation inactivante n'a été découverte au niveau des gènes *hMLH1* et *hMSH3.*

Ensuite, nous avons démontré que le promoteur d'*hMLH1* était méthylé dans 75% des cas exprimant un phénotype mutateur. Ce résultat renforce l'hypothèse que l'hyperméthylation de régions riches en CpG associées au promoteur est un important mécanisme d'inactivation des gènes de réparation de mésappariements de bases de l'ADN dans les tumeurs 'instables' (Kane *et al.,* 1997). L'implication d'une méthylation aberrante dans les cancers a aussi été rapportée pour des gènes impliqués dans la régulation de la croissance cellulaire tels que les gènes suppresseurs (revue dans Jones, 1996 ; Greger *et al.,* 1989 ; Sakai *et al.,* 1991; Herman *et al.,* 1994 ; Merlo *et al.,* 1995 ; Herman *et al.,* 1995 ; Gonzales-Zulueta *et al.,* 1995 ; Graff *et al.,* 1995 ; Prowse *et al.,* 1997). S'il est vrai que la méthylation du promoteur est un mécanisme d'inactivation de gène qui joue un rôle dans le développement du cancer, ceci aura des implications cliniques importantes concernant la possibilité de réactiver ces gènes à travers l'utilisation d'inhibiteurs de la méthylation de l'ADN (Jones, 1996).

CONCLUSIONS

Dans cette étude d'allélotypage nous avons démontré : (1) un phénotype mutateur est présent dans les cas sporadiques de cancer du côlon, mais est pratiquement absent des tumeurs sporadiques du sein et du poumon ; (2) les loci *hMLH1* et *hMSH3* sont délétés de façon hémizygote dans ces cancers et joueraient un rôle via des fonctions cellulaires autres que la réparation d'erreurs commises lors de la réplication ; (3) aucune corrélation n'a été trouvée entre la présence de PDH et l'expression d'un phénotype mutateur dans ces trois tumeurs ; (4) la méthylation du promoteur de *hMLH1* suggère un mécanisme alternatif d'inactivation des gènes SRM dans les cas de cancer du côlon exprimant un phénotype mutateur.

BIBLIOGRAPHIE

Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, Jarniven H, Powell SM, Jen J, Hamilton SR, Peterson GM, Kinzler KW, Vogelstein B, de la Chapelle A (1993) Clues to the pathogenesis of familial colorectal cancer. *Science* **260**:812-816

Acharya S, Wilson T, Gradia S, Kane MF, Guerrette S, Marsischky GT, Kolodner RD, Fishel R (1996) hMSH2 forms specific mispair-binding complexes with hMSH3 and hMSH6. *Proc. Natl. Acad. Sci. USA* **93**:13629-13634

Ahuja N, Mohan AL, Li Q, Stolker JM, Herman JG, Hamilton SR, Baylin SB, Issa JP (1997) Association between CpG island methylation and microsatellite instability in colorectal cancer. *Cancer Res.* **57**:3370-3374

Anderson TI (1996) Genetic heterogeneity in breast cancer susceptibility. *Acta Oncologica* **35**:407-410

Ashton-Rickart PG, Wyllie AH, Bird CC, Dunlop MG, Steel CM, Morris RG, Piris J, Romanowski P, Wood R, White R, Nakamura Y (1991) MCC, a candidate familial polyposis gene in 5q21 shows frequent allele loss in colorectal and lung cancer. *Oncogene* **6**:1881-1886

Baccichet A, Qualman SK, Sinnett D (1997) Allelic loss in childhood acute lymphoblastic leukemia. *Leuk. Res.* **21**:817-823

Baker SM, Bronner CE, Zhang L, Plug AW, Robatzek M, Warren G, Elliott EA, Yu J, Ashley T, Arnheim N, Flavell RA and Liskay RM (1995) Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis. *Cell* **82**:309-320

Barnes LD, Garrison PN, Siprashvili Z, Guranowski A, Robinson AK, Ingram SW, Croce CM, Ohta M, Huebner K (1996) Fhit, a putative tumor suppressor in humans, is a dinucleoside 5', 5'''-P1,P3 triphosphate hydrolase. *Biochemistry* **35**:11529-11535

Benachenhou N, Guiral S, Gorska-Flipot I, Labuda D, Sinnett D (1998a) High resolution deletion mapping reveals frequent allelic losses at the DNA mismatch repair loci hMLH1 and hMSH3 in non-small cell lung cancer. *Int. J. Cancer* **77**: 173-180

Benachenhou N, Guiral S, Gorska-Flipot I, Labuda D, Sinnett D (1998b) Frequent loss of heterozygosity at the DNA mismatch-repair loci hMLH1 and hMSH3 in sporadic breast cancer. *Br. J. Cancer* (in press)

Benachenhou N, Guiral S, Gorska-Flipot I, Michalski R, Labuda D, Sinnett D (1998c) Allelic losses and DNA methylation at DNA mismatch repair loci in sporadic colorectal cancer. *Carcinogenesis* (in press)

Bièche I, Lidereau R (1995) Genetic alterations in breast cancer. *Genes Chromosom. Cancer* **14**: 227-251

Boveri T, *The origin of malignant tumors*, pp.26-27, Williams and Wilkins, Baltimore, 1929

Boynton RF, Blount PL, Yin J, Brown VL, Huang Y, Tong Y, McDaniel T, Newkirk C, Resau JH (1992) Loss of heterozygosity involving the APC and MCC genetic loci occurs in the majority of human esophageal cancers. *Proc. Natl. Acad. Sci. USA* **89**:3385-3388

Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A, Tannergard P, Bollag RJ, Godwin AR, Ward DC, Nordenskjold M, Fishel R, Kolodner R, Liskay RM (1994) Mutation in the DNA mismatch repair gene homolog hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature* **368**:258-261

Carney DN, DeLeij L (1988) Lung cancer biology. *Semin. Oncol.* **15**:199

Cavenee WK, Dryja TP, Philips RA, Benedict WF, Godbout R, Gallie BL, Murphree AC, Strong LC, White R (1983) Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. *Nature* **305**:779-784

Cavenee WK, Hansen MF, Nordenskjöld M, Kock E, Maumenee I, Squire JA, Philips RA, Gallie BL (1985) Genetic origin of mutations predisposing to retinoblastoma. *Science* **228**:501-503

Cho K, Vogelstein, B (1991) Genetic alterations in the adenoma-carcinoma sequence. *Cancer* **6 (Suppl)** : 1727-1731

Chen LC, Kurisu W, Ljung BM, Goldman ES, Moore D 2d, Smith HS (1992) Heterogeneity for allelic loss in human breast cancer. *J. Natl. Cancer Inst.*. **84**:506-510

Chen LC, Matsumura K, Deng G, Kurisu W, Ljung BM, Lerman MI, Waldman FM, Smith HS (1994) Deletion of two separate regions on chromosome 3p in breast cancers. *Cancer Res.* **54**:3021-3024

Cooper GM, *Oncogenes* , second edition, Jones and Bartlett, Boston, 1995

Cornelisse CJ, Kuipers-Dijkshoorn N, van Vliet M, Hermans J, Devilee P (1992) Fractional allelic imbalance in human breast cancer increases with tetraploidization and chromosome loss. *Int. J. Cancer* **50**:544-548

Daly MC, Xiang RH, Buchhagen D, Hensel CH, Garcia DK, Killary AM, Minna J, Naylor SL (1993) A homozygous deletion on chromosome 3 in a small cell lung cancer cell line correlates with a region of tumor suppressor activity. *Oncogene* **8**:1721-1729

D'amico D, Carbone DP, Johnson BE, Meltzer J, Minna JD (1992) Polymorphic sites within the MCC and APC loci reveal very frequent loss of heterozygosity in human small cell lung cancer. *Cancer Res.* **52**:1996-1999

DeWeese TL, Bucci JM, Larrier NA, Cutler RG, te Riele H, Nelson WG (1997) Tolerance of oxidative DNA damage results from disruption of *Msh2* alleles: implications for carcinogenesis. Proceedings, 88th Annual Meeting American Association for Cancer Research, vol. 38.

deWind N, Dekker M, Berns A, Radman M, te Riele H (1995) Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell* **82**:321-330

Drummond JT, Li GM, Longley MJ, Modrich P (1995) Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. *Science* **268**:1909-1912

Fishel R, Lescoe MK, Rao MRS, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* **75**:1027-1038

Fishel R, Ewell A, Lee S, Lescoe MK, Griffith J (1994a) Binding of mismatched microsatellite DNA sequence by the human MSH2 protein. *Science* **266**:1403-1405

Fishel R, Ewell A, Lescoe MK (1994b) Purified human MHS2 protein binds to DNA containing mismatched nucleotides. *Cancer Res.* **54**:5539-5542

Fishel R, Kolodner D (1995) Identification of mismatch repair genes and their role in the development of cancer. *Curr. Opin. Genet. Dev.* **5**:382-395

Fishel R, Wison T (1997) MutS homologs in mammalian cells. *Curr. Opin. Genet. Dev.* 7:105-113

Fong, KM, Zimmerman, PV and Smith, PJ, (1995) Microsatellite instability and other molecular abnormality in non-small cell lung cancer. *Cancer Res* **55**:28-30

Friedberg EC, Bardwell AJ, Bardwell L, Wang Z, Dianov G (1994) Transcription and nucleotide excision repair – reflections, considerations and recent biochemical insights. *Mut. Res.* **307**:5-14

Friedberg EC (1996) Relationship between DNA repair and transcription. *Annu. Rev. Biochem.* **65**:15-42

Gonzalez-Zulueta M, Bender CM, Yang AS, Nguyen T, Beart RW, Van Tornout JM and Jones P (1995) Methylation of the 5' CpG island of the p16/CDKN2 tumor suppressor gene in normal and transformed human tissues correlates with gene silencing. *Cancer Res.* **55**:4531-4535

Gradia S, Acharya S, Fishel R (1997) The human mismatch recognition complex hMSH2-hMSH6 functions as a novel molecular switch. *Cell* **91**:995-1105

Graff JR, Herman JG, Lapidus RG, Chopra H, Xu R, Jarrard DF, Isaacs WB, Pitha PM, Davidson NE and Baylin SB (1995) E-cadherin expression is silenced by DNA hypermethylation in human breast and prostate carcinomas. *Cancer Res.* **55**:5195-5199

Greger V, Passarge E, Hopping W, Messmer E and Horsthemke B (1989) Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. *Hum. Genet.* **83**:155-158

Habraken JA, Sung P, Prakash L, Prakash S (1996) Binding of insertion/deletion DNA mismatches by the heterodimer of yeast mismatch repair proteins MSH2 and MSH3. *Curr. Biol.* **6**:1185-1187

Hackman P, Gabbani G, Österholm AM, Hellgren D, Lambert B (1995) Spontaneous lenght variation in microsatellite DNA from human T-cell clones. *Genes Chromosom. Cancer* **14**:215-219

Han HJ, Yanagisawa A, Kato Y, Park JG, Nakamura Y (1993) Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. *Cancer Res.* **53**:5087-5089

Hartwell L (1992) Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. *Cell* **71**:543-546.

Hawn MT, Umar A, Carethers JM, Marra G, Kunkel TA, Boland CR, Koi M (1995) Evidence for a connection between the mismatch repair system and the G2 cell cycle checkpoint. *Cancer Res.* **55**:3721-3725

Helzlsouer KJ, Harris EL, Parshad R, Perry HR, Price FM, Sanford KK (1996) DNA repair proficiency: Potential susceptibility factor for breast cancer. *J. Natl. Cancer Inst.* **88**:754-755

Hemminki KJ, Peltomaki P, Mecklin JP, Jarvinen H, Salvaara R, Nystrom-Lahti M, de la Chapelle A, Aaltonen LA (1994) Loss of the wild-type *MLH1* gene is a feature of hereditary nonpolyposis colorectal cancer. *Nat. Genet.* **8**:405-410

Herman JG, Latif F, Weng Y Lerman MI, Zbar B, Liu S, Samid D, Duan DS, Gnarra JR, Linehan WM, Baylin SB (1994) Silencing of the VHL tumor-suppressor gene by DNA methylation in renal cell carcinoma. *Proc. Natl. Acad. Sci. USA* **91**:9700-9704

Herman JG, Merlo A, Mao L, Lapidus RG, Issa JP, Davidson NE, Sidransky D, Baylin SB (1995) Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. *Cancer Res.* **55**:4525-4530

Institut national du Cancer du Canada, statistiques canadiennes sur le cancer, 1998, Toronto, Canada, 1998

Ionov Y, Peinado A, Malkhosyan S, Shibata D, Perucho M (1993) Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colon carcinogenesis. *Nature* **363**:558-561

Jäger AC, Bisgaard ML, Myrhøj T, Bernstein I, Rehfeld JF and Nielsen FC (1997) Reduced frequency of extracolonic cancers in hereditary nonpolyposis colorectal cancer families with monoallelic hMLH1 expression. *Am. J. Hum. Genet.* **61**:129-138

Jones PA (1996) DNA methylation errors and cancer. *Cancer Res.* **56**:2463-2467

Kane MF, Loda M, Gaida G.M, Lipman J, Mishra R, Goldman H, Jessup JM, Kolodner R, (1997) Methylation of the *hMLH1* promoter correlates with lack of expression of *hMLH1* in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res.* **57**:808-811.

Karnik P, Plummer S, Casey G, Myles J, Tubbs R, Crowe J, Williams BR (1995) Microsatellite instability at a single locus (*D11S988*) on chromosome 11p15.5 as a late event in mammary tumorigenesis. *Hum. Mol. Genet.* **4**:1889-1894

Kat A, Thilly WG, Fang WH, Longley MJ, Li GM, Modrich P (1993) An alkylation-tolerant, mutator human cell line is deficient in strand-specific mismatch repair. *Proc. Natl. Acad. Sci. USA* **90**:6424-6428

Kinzler KW, Vogelstein B (1997) Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature* **386**:761

Knudson AG Jr (1971) Mutation and cancer : statistical study of retinoblastoma. *Proc. Natl. Acad. Sci. USA* **68**:820-823

Knudson AG Jr (1993) Antioncogenes and human cancer. *Proc. Natl. Acad. Sci. USA* **90**:10914-10921

Kok K, Osinga J, Carritt B, Davis MB, van der Hout AH, van der Veen AY, Landsvater RM, de Leij LF, Berendsen HH, Postmus PE, Poppema S, Buys CHCM (1987) Deletion of a DNA sequence at the chromosomal region 3p21 in all major types of lung cancer. *Nature* **330**:578-581

Kok K, Naylor SL and Buys CH, (1997) Deletions of the short arm of chromosome 3 in solid tumors and the search for suppressor genes. *Adv. Cancer Res.* **71**:27-92

Kolodner R, Alani E (1994) Mismatch repair and cancer susceptibility. *Curr. Opin. Biotech.* **5**:585-594

Kolodner RD, Hall NR, Lipford JR, Kane MF, Rao MRS, Morrison P, Wirth L, Finan PJ, Burn J, Chapman P (1994) Structure of the human MSH2 locus and analysis of two Muir-Torre kindreds for msh2 mutations. *Genomics* **24**:516-526

Kolodner RD, Hall NR, Lipford J, Kane MF, Morrison PT, Finan PJ, Burn J, Chapman P, Earabino C, Merchant E, Bishop DT (1995) Structure of the MLH1 locus and analysis of HNPCC kindreds for MLH1 mutations. *Cancer Res.* **5**:242-248.

Kolodner RD (1996) Biochemistry and genetics of eukaryotic mismatch repair. *Genes Dev.* **10**:1433-1442

Kunkel Ta (1995) DNA mismatch repair, the intricacies of eukaryotic spell-checking. *Curr. Biol.* **5**:1091-1094

LaForgia SK, Morse B, Levy J, Barnea G, Li F, Cannizarro LA, Nowell PC, Glick J, Boghosian-Sell L, Weston A, Harris CC, Drabkin H, Patterson D, Croce CM, Schlessinger J, Huebner K (1991) Receptor protein-tyrosine phosphatase is a candidate tumor suppressor gene at human chromosome region 3p21. *Proc. Natl. Acad. Sci. USA* **88**:5036-5040

Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, Peltomaki P, Sistonen P, Aaltonen LA, Nystrom-Lahti M, Guan XY, Zhang J, Meltzer PS, Yu JW, Kao FT, Chen DJ, Cerosaletti KM, Fournier REK, Todd S, Lewis T, Leach RJ, Naylor SL, Weissenbach J, Mecklin JP, Järvinen H, Petersen GM, Hamilton SR, Green J, Jass J, Watson P, Lynch TT, Trent JM, de la Chapelle A, Kinzler KW, Vogelstein B (1993) Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* **75**:1215-1225

Lengauer C, Kinzler KW and Vogelstein B (1997) Genetic instability in colorectal cancers. *Nature* **386**:623-627

Levine AJ (1993) The tumor suppressor genes. *Annu. Rev. Biochem.* **62**:623-657

Levinso G, Gutman GA (1987) High frequencies of short frameshifts in poly-CA/TG tandem repeats borne by bacteriophage M13 in *Escherichi coli* K-12. *Nucleic Acids Res.* **15**:5323-5338

Li GM, Modrich P (1995) Restoration of mismatch repair to nuclear extracts of H6 colorectal tumor cells by a heterodimer of human MutL homologs. *Proc. Natl. Acad. Sci. USA* **92**:1950-1954

Liu B, Nicolaides NC, Markowithz S, Willson KV, Parsons RE, Jen J, Papadopoulos N, Peltomaki P, de la Chapelle A, Hamilton, JR, Kinzler KW, Vogelstein B (1995) Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. *Nat. Genet.* **9**:48-55

Liu B, Parsons R, Papadopoulos N, Nicolaides NC, Lynch HT, Watson P, Jass JR, Dunlop M, Wyllie A, Peltomaki P, dela Chapelle A, Hamilton SR, Vogelstein B, Kinzler KW (1996) Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. *Nat. Med.* **2** : 1169-174

Loeb LA (1991) Mutator phenotype may be required for multistage carcinogenesis. *Cancer Res.* **51**:3075-3079

Lothe RA, Peltomaki P, Meling GI, Aaltonen LA, Nystrom-Lahti M, Pylkkanen L, Heimdal K, Andersen TI, Moller P, Rognum TO, Fossa SD, Haldorsen T, Langmark F, Brogger A, de la Chapelle A, Borresen AL (1993) Genomic instability in colorectal cancer: Relationship to clinicopathological variables and family history. *Cancer Res.* **53**:5849-5852

Lynch HT, Smyrk TC, Watson P, Lanspa SJ, Lynch JF, Lynch PM, Cavalieri RJ, Boland CR (1993) Genetics, natural history, tumor spectrum and pathology of hereditary

nonpolyposis colorectal cancer : an updated review. *Gastroenterology* **104**:1535-1539

Marsischky GT, Filosi N, Kane MF, Kolodner R (1996) Redundancy of *Saccharomyces cerevisae* MSH3 and MSH6 in MSH2-dependent mismatch repair. *Genes Dev.* **10**:407-420

Matsumoto S, Kasumi F, Sakamoto G, Onda M, Nakamura Y, Emi M (1997) Detailed deletion mapping of chromosome arm 3p in breast cancers : a 2-cM region on 3p14.3-21.1 and 5-cM region on 3p24.3-25.1 commonly deleted in tumors. *Genes Chromosom. Cancer* **20**:268-274

Medeiros AC, Nagai MA, Neto MM, Brentani RR (1994) Loss of heterozygosity affecting the APC and MCC genetic loci in patients with primary breast carcinomas. *Cancer Epidemiol. Biomarkers Prev.* **3**:331-333

Mello JA, Acharya S, Fishel R, Essigmann JM (1996) The mismatch-repair protein hMSH2 binds selectively to DNA adducts of the anticancer drug cisplatin. *J. Chem. Biol.* **3**:579-589

Mellon I, Rajpal DK, Koi M, Boland CR, Champe N (1996) Transcription-coupled deficiency and mutations in mismatch repair genes. *Science* **272**:557-560

Merlo A, Mabry M, Gabrielson E, Vollmer R, Baylin SB, Sidransky D (1994) Frequent microsatellite instability in primary small cell lung cancer. *Cancer Res.* **54**:2098-2101

Merlo A, Herman JG, Mao L, Lee DJ, Gabrielson T, Burger PC, Baylin SB, Sidransky D (1995) 5' CpG island methylation is associated with transcriptional silencing of the tumor suppressor p16/CDKN2/MTS1 in human cancers. *Nat. Med.* **1**:686-692

Modrich P (1991) Mechanisms and biological effects of mismatch repair. *Annu. Rev. Genet.* **25**:229-253

Modrich P, Lahue R (1996) Mismatch repair in replication fidelity, genetic recombination and cancer biology. *Annu. Rev. Biochem.* **65**:101-133

Mu D, Turson M, Duckett DR, Drummond JT, Modrich P, Sancar A (1997) Recognition and repair of compound DNA lesions (base damage and mismatch) by human mismatch repair and excision repair systems. *Mol. Cell Biol.* **17**:760-769

Muller JP, Smerdon MJ (1996) Rad23 is required for TCR and efficient overall repair in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **16**:2361-2368

Nakajima E, Orimo H, Ikejima M, Shimada T (1995) Nine-bp repeat polymorphism in exon 1 of the *hMSH3* gene. *Jpn. J. Hum. Genet.* **40**:343-345.

Naylor SL, Johnson BE, Minna JD, Sakaguchi AY (1987) Loss of heterozygosity of chromosome 3p markers in small-cell lung cancer. *Nature* **329**:451-454

Negrini M, Monaco C, Vorechovsky I, Ohta M, Druck T, Baffa R, Huebner K, Croce CM (1996) The FHIT gene at 3p14.2 is abnormal in breast carcinomas. *Cancer Res.* **56**:3173-3179

Nicolaides NC, Papadopoulos N, Liu B, Wei Y, Carter KC, Ruben SM, Rosen CA, Haseltine WA, Fleischman RD, Fraser CM, Adams MD, Venter JC, Dunlop MG, Hamilton SR, Peterson M, de la Chapelle A, Vogelstein B, Kinzler KW (1994) Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature* **371**:75-80

Nowell PC (1976) The clonal evolution of tumor cell populations. *Science* **194**:23-28

Ohta M, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, Siprashvili Z, Mori M, McCue P, Druck T, Croce CM, Huebner K (1996) The human FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma associated translocation breakpoint, is abnormal in digestive tract cancers. *Cell* **84**:587-597

Palombo F, Iaccarino I, Nakajima E, Ikejima M, Shimada T, Jiricny J (1996) hMutSbeta, a heterodimer of hMSH2 and hMSH3, binds to insertion/deletion loops in DNA. *Curr. Biol.* **6**:1181-1184

Pandis N, Jin Y, Limon J, Bardi G, Idvall I, Mandahl N, Mitelman F, Heim S (1993) Interstitial deletion of the short arm of chromosome 3 as a primary chromosome abnormality in carcinomas of the breast. *Genes Chromosom. Cancer* **6**:151-155

Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen C.A., Haseltine WA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Hamilton SR, Petersen GM, Watson P, Lynch HT, Peltomäki P, Mecklin JP, de la Chapelle A, Kinzler KW, Vogelstein B (1994) Mutation of a mutL homolog in hereditary colon cancer. *Science* **263**:1625-1629

Parshad R, Price FM, Bohr VA, Cowans KH, Zujewski JA, Sanford KK (1996) Deficient DNA repair capacity, a predisposing factor in breast cancer. *Br. J. Cancer* **74**:1-5

Parsons R, Li GM, Longly MJ, Fang W, Papadopoulos N, Jen J, de la Chapelle A, Kinzler KW, Vogelstein B, Modrich P (1993) Hypermutability and mismatch repair deficiency in RER+ tumor cells. *Cell* **75**:1227-1236

Paulson TJ, Wrught FA, Parker BA, Russack V, Walh GM (1996) Microsatellite instability correlates with reduced survival and poor disease prognosis in breast cancer. *Cancer Res.* **56**:4021-4026

Peltomaki P, Aaltonen LA, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Green JS, Jass JR, Weber JL, Leach FS, Peterson GM, Hamilton SR, de la Chapelle A, Vogelstein B (1993) Genetic mapping of a locus predisposing to human colorectal cancer. *Science* **260**:810-812

Peltomaki P, Vasen HF, and the International Collaborative Group on HNPCC (1997) Mutations predisposing to hereditary nonpolyposis colorectal cancer : database and results of a collaborative study. *Gastroenterology* **113:**1146-1158

Peltomaki P, de la Chapelle A (1997) Mutations predisposing to hereditary nonpolyposis colorectal cancer. *Adv. Cancer Res.* **71**:93-119

Petersen I, Langreck H, Wolf G, Schwendel A, Psille R, Vogt P, Reichel MB, Ried T, Dietel M (1997) Small-cell lung cancer is characterized by a high incidence of deletions on chromosomes 3p, 4q, 5q, 10q, 13q and 17p. *Br. J. Cancer* **75**:79-86

Pifarre A, Rosell R, Monzo M, De Anta JM, Moreno I, Sanchez JJ, Ariza A, Mate JL, Martinez E, Sanchez M (1997) Prognostic value of replication errors on chromosomes 2p and 3p in non-small cell lung cancer. *Br. J. Cancer* **75**:184-189

Prolla TA, Pang Q, Alani E, Kolodner RD, Liskay RM (1994) Interractions between the MSH2, MLH1 and PMS1 proteins during the initiation of DNA mismatch repair. *Science* **265**:1091-1093

Prowse AH, Webster AR, Richards FM, Richard S, Olschwang S, Resche F, Affara NA, Maher ER (1997) Somatic inactivation of the VHL gene in von Hippel-Lindau disease tumors. *Am. J. Hum. Genet.* **60**:765-771

Rabbitts P, Koga H, Takahashi R, Figlin RA, Holmes EC, Piantadosi S, Cordon-Cardo C, Slamon DJ (1989) Frequency and extent of allelic loss in the short arm of chromosome 3 in non-small cell lung cancer. *Genes Chromosom. Cancer* **1**:95-105

Remvikos Y, Vogt N, Muleris M, Salmon RJ, Malfoy B and Dutrillaux B (1995) DNA-repeat instability is associated with colorectal cancers presenting minimal chromosome rearrangements. *Genes Chromosom. Cancer* **12**:272-276

Roche J, Boldog F, Robinson M, Robinson L, Varella-Garcia M, Swanton,M, Waggoner B, Fishel, R Franklin W, Gemmill R, Drabkin H (1996) Distinct 3p21.3 deletions in lung cancer and identification of a new human semaphorin. *Oncogene* **12**:1289-1297

Sakai T, Toguchida J, Ohtani N, Yandell DW, Rapaport JM, Dryja TP (1991) Allele-specific hypermethylation of the retinoblastoma tumor suppressor gene. *Am. J. Hum. Genet.* **48**:880-888

Sato T, Tanigami A, Yamakawa K, Akiyama F, Kasumi F, Sakamoto G, Nakamura Y (1990) Allelotype of breast cancer : Cumulative allele losses promote tumor progression in primary breast cancer. *Cancer Res.* **50**:7184-7189

Shridhar V, Siegfried J, Hunt J, del Mar Alonso M, Smith DI (1994) Genetic instability of microsatellite sequences in many non-small cell lung carcinomas. *Cancer Res.* **54**:2084-2087

Sia EA, Kokosku RJ, Dominska M, Greewell P, Petes TD (1997) Microsatellite instability in yeast : dependence on repeat unit size and DNA mismatch repair genes. *Mol. Cell. Biol.* **17**:2851-2858

Solomon E, Voss R, Hall V, Bodmer WF, Jass JR, Jeffreys AJ, Lucibello FC, Patel I, Rider SH (1987) Chromosome 5 allele loss in human colorectal carcinomas. *Nature* **328**:616-619

Sorio C, Mendrola J, Lou Z, LaForgia S, Croce CM, Huebner K (1995) Characterization of the receptor protein tyrosine phosphatase gene product PTP gamma : binding activation by triphosphorylated nucleosides. *Cancer Res.* **55**:4855-4864

Speicher MR (1995) Microsatellite instability in human cancer. *Oncol. Res.* 7:267-275

Strand M, Prolla TA, Liskay RM, Petes TP (1993) Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* **365**:274-276

Tavassoli M, Steingrimsdottir H, Pierce E, Jiang X, Alagoz M, Farzaneh F, Campbell IG (1996) Loss of heterozygosity on chromosome 5q in ovarian cancer is frequently accompanied by TP53 mutation and identifies a tumor suppressor gene locus at 5q13.1-21. *Br. J. Cancer* **74**:115-119

Thibodeau SN, Bren G and Schaid D (1993) Microsatellite instability in cancer of the proximal colon. *Science* **260**:816-819

Tomlinson IP, Ilyas M, Bodmer WF (1996) Allele loss occurs frequently at *hMLH1*, but rarely at *hMSH2*, in sporadic colorectal cancers with microsatellite instability. *Br. J. Cancer* **74**:1514-1517

Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL (1988) Genetic alterations during colorectal-tumor development. *N. Engl. J. Med.* **319**:525-532

Vogelstein B, Fearon ER, Kern SE, Hamilton SR, Preisinger AC, Nakamura Y, White R (1989) Allelotype of colorectal carcinomas. *Science* **244**:207-211

Vogelstein B, Kinzler KW (1993) The multistep nature of cancer. *Trends in Genetics* **4** : 138-141

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* **44**:388-396

Wei, Q, Cheng, L, Hog, WK, and Spitz, MR (1995) Reduced DNA repair capacity in lung cancer patients. *Cancer Res.* **56**:4103-4107

Wieland I, Ammermuller T, Bohm M, Totzeck B, Rajewsky MF (1996 Microsatellite instability and loss of heterozygosity at the *hMLH1* locus on chromosome 3p21 occur in a subset of non-small cell lung carcinomas. *Oncol. Res.* **8**:1-5

Wood RD (1996) DNA repair in eukaryotes. *Annu. Rev. Biochem.* **65**:135-167

Wooster R, Cleton-Jansen AM, Collins N, Mangion J, Cornelis RS, Cooper CS, Gusterson BA, Ponder BA, von Deimling A, Wiestler OD, Cornelisse CJ, Devilee P, Stratton MR (1994) Instability of short tandem repeats (microsatellites) in human cancers. *Nat. Genet.* **6**:152-156

Yokota J, Sugimura T (1993) Mutiple steps in carcinogenesis involving alterations of multiple tumor suppressor genes. *FASEB J.* **7** : 920-925

Yee CJ, Roodi N, Verrier CS, Parl FF (1994) Microsatellite instability and loss of heterozygosity in breast cancer. *Cancer Res.* **54**:1641-1644

Sébastien Guiral

Curriculum Vitae

Formation Académique

1996-1998 **MAÎTRISE EN SCIENCES, PROGRAMME DE BIOLOGIE MOLÉCULAIRE**
Directeur de recherche : Daniel Sinnett, Ph.D.
Université de Montréal, Centre de Cancérologie Charles Bruneau, Hôpital Sainte-Justine, Montréal, Québec

PROJET DE RECHERCHE
Allélotypage des gènes de réparation de l'ADN dans les cancers non-héréditaires

1993-1996 **BACCALAURÉAT EN SCIENCES AVEC SPÉCIALISATION EN BIOCHIMIE** (First Class Honours)
Université McGill, Montréal, Québec

Expérience de travail reliée au domaine

ÉTÉ 1996 **STAGIAIRE DE RECHERCHE**
Laboratoire du Dr. Daniel Sinnett, Centre de Cancérologie Charles Bruneau, Hôpital Sainte-Justine, Montréal, Québec

Divers

- Gagnant du 1er prix pour les présentations de posters (Maîtrise) au XIIIème Congrès Annuel de la Recherche des étudiants gradués du Centre de Recherche de l'Hôpital Sainte-Justine
- Boursier du FRSQ/FCAR
- Vice-président de l'Association des Étudiants Gradués de l'Hôpital Sainte-Justine
- Félicité par le département pour ma performance académique dans le programme de 'Honours Biochemistry' à l'Université McGill
- Sélection à 'l'Academic Honour Roll' au CEGEP John Abbott College

Publications

Articles

1) N. Benachenhou*, **S. Guiral***, I. Görska-Flipot, D. Labuda et D. Sinnett (1998) High resolution deletion mapping reveals frequent allelic losses at the DNA mismatch-repair loci *hMLH1* and *hMSH3* in non-small cell lung cancer. Int. J. Cancer : *77*, 173-180. **N.B. et S.G. ont contribué de façon équivalente à ce travail.**

2) N. Benachenhou, **S. Guiral**, I. Görska-Flipot, D .Labuda et D. Sinnett (1998) Frequent loss of heterozygosity at the DNA mismatch-repair loci *hMLH1* and *hMSH3* in sporadic breast cancer (Sous presse dans le *British Journal of Cancer*).

3) N. Benachenhou*, **S. Guiral***, I. Görska-Flipot, R. Michalski, D. Labuda et D. Sinnett (1998) Allelic losses and DNA methylation at DNA mismatch repair loci in sporadic colorectal cancer. (Sous presse dans *Carcinogenesis*). * **N.B. et S.G. ont contribué de façon équivalente à ce travail.**

4) **S. Guiral**, N. Benachenhou, D. Labuda et D. Sinnett (1998) Allelotyping analysis of DNA mismatch-repair loci reveals LOH at *hPMS2* in childhood acute lymphoblastic leukemia (en préparation).

Communications

1) **S. Guiral**, N. Benachenhou, M. Krajinovic, D. Labuda et D. Sinnett (1998) Frequent loss of heterozygosity at the DNA mismatch-repair loci *hMLH1* and *hMSH3* in sporadic breast cancer and non-small cell lung cancer. XIIIème Congrès Annuel de la Recherche des étudiants gradués du Centre de Recherche de l'Hôpital Sainte-Justine, 5 Juin, 1998, Montréal, Québec

2) **S. Guiral**, N. Benachenhou, M. Krajinovic, D. Labuda et D. Sinnett (1998) Frequent loss of heterozygosity at the DNA mismatch-repair loci *hMLH1* and *hMSH3* in sporadic breast cancer and non-small cell lung cancer. 89th Annual Meeting, American Association for Cancer Research, 28 Mars-1 Avril, 1998, New Orleans, Louisiane

3) N. Benachenhou, D. Labuda , **S. Guiral** et D. Sinnett (1997) Frequent loss of heterozygosity at the DNA mismatch-repair loci *hMLH1* and *hMSH3* in sporadic breast cancer. 2nd World Congress on Advances in Oncology, 16-18 Octobre, 1997, Athènes, Grèce

Communications (suites)

4) **S. Guiral**, N. Benachenhou, D. Labuda et D. Sinnett (1997) Frequent loss of heterozygosity at the DNA mismatch-repair loci *hMLH1* and *hMSH3* in sporadic breast cancer. XIIème Congrès Annuel de la Recherche des étudiants gradués du Centre de Recherche de l'Hôpital Sainte-Justine, 6 Juin, 1997, Montréal, Québec
5) D. Labuda , N. Benachenhou, **S. Guiral** et D. Sinnett (1997) Frequent loss of heterozygosity at the DNA mismatch-repair loci *hMLH1* and *hMSH3* in sporadic breast cancer. Terry Fox Workshop on Cancer Genetics, 31 Mai-1er Juin 1997, Toronto, Ontario
6) **S. Guiral**, N. Benachenhou, D. Sinnett et D. Labuda (1997) Frequent loss of heterozygosity at the DNA mismatch-repair loci in sporadic breast cancer. IIIe Journée scientifique du programme de Biologie Moléculaire, 16 Mai, 1997, Montréal, Québec
7) **S. Guiral**, N. Benachenhou, I. Görska-Flipot, D. Sinnett et D. Labuda (1997) Allelotyping of DNA mismatch-repair loci in sporadic breast cancer. Human Genome Meeting, 6-8 Mars, 1997, Toronto, Ontario