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ORIGINAL ARTICLE

Promoter hypermethylation of DNA repair genes MLH1 and MSH2 in adenocarcinomas and squamous cell carcinomas of the lung

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Abstract Five years survival of lung cancer is 16%, significantly lower than in prostate (99.9%), breast (88.5%) and colon (64.1%) carcinomas. When diagnosed in the surgical stage it increases to 50% but this group only comprises 14–16% of the cases. DNA methylation has emerged as a potential cancer-specific biomarker. Hypermethylation of CpG islands located in the promoter regions of tumour suppressor genes is now firmly established as an important mechanism for gene inactivation.

This retrospective study included 40 squamous cell carcinomas and 40 adenocarcinomas in various surgical TNM stages to define methylation profile and possible silencing of DNA repair genes – *MLH1* and *MSH2* – using Methylation-Specific PCR and protein expression by immunohistochemistry in tumoural tissue, preneoplastic lesions and respiratory epithelium with normal histological features.

The protein expression of *MLH1* and *MSH2* genes, in the available preneoplastic lesions and in normal cylindrical respiratory epithelium appeared reduced. The frequency of promoter hypermethylation found on these DNA repair genes was elevated, with a higher prevalence of methylation of *MLH1* gene in 72% of squamous cell carcinoma. The differences are not so obvious for *MSH2* promoter hypermethylation. No correlation was found among the status of methylation, the protein expression and the clinicopathological characteristics.

With a larger study, a better characterization of the hypermethylation status of neoplastic and preneoplastic lesions in small biopsies would be achieved, inherent to tumour histology, heterogeneity and preservation, and finally differences in the study population to elucidate other possible mechanisms of altered expression of the *hMLH1* and *hMSH*.

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PALAVRAS CHAVE
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Hipermetilação Promotora de Genes Reparadores de DNA MLH1 e MSH2 em Adenocarcinomas e Carcinomas de Células Escamosas do Pulmão

Resumo A sobrevivência aos cinco anos no cancro do pulmão é de 16%, significativamente inferior que nos carcinomas na próstata (99,9%), mama (88,5%) e cólon (64,1%). Quando diagnosticado na fase cirúrgica aumenta até 50%, mas este grupo é apenas constituído por 14-16% dos casos. A metilação do ADN surgiu como um potencial marcador biológico específico do cancro. A hipermetilação das ilhas CpG localizadas nas regiões promotoras de genes supressores do tumor está agora firmemente estabelecida como um mecanismo importante para a inativação do gene.

Este estudo retrospetivo incluiu 40 carcinomas das células escamosas e 40 adenocarcinomas em vários estádios cirúrgicos TNM para definir o perfil da metilação e o possível silenciamento de genes de reparação do ADN - *MLH1* e *MSH2* - usando metilação PCR específica e expressão da proteína por imuno-histoquímica no tecido tumoral, lesões pré-neoplásicas e epitélio respiratório com características histológicas normais.

A expressão da proteína dos genes *MLH1* e *MSH2*, nas lesões pré-neoplásticas disponíveis e no epitélio respiratório cilíndrico normal, pareceu reduzida. A frequência da hipermetilação promotora encontrada nestes genes reparadores de ADN foi elevada, com uma maior prevalência da metilação do gene *MLH1* em 72% de carcinoma de células escamosas. As diferenças não são tão óbvias para a hipermetilação do promotor *MSH2*. Não foi encontrada correlação entre o estado de metilação, a expressão da proteína e as características clínico-patológicas.

Com um estudo mais amplo, seria alcançada uma melhor caracterização do estado da hipermetilação das lesões neoplásicas e pré-neoplásicas em pequenas biopsias, inerente à histologia, heterogeneidade e preservação do tumor, e, finalmente, às diferenças na população estudada para elucidar outros mecanismos possíveis da expressão alterada do *hMLH1* e *hMSH*.

Introduction

Epigenetics of human cancer has been overshadowed by human cancer genetics since 1983. Increasingly visible with a growing understanding of specific epigenetic mechanisms and their role in cancer,[1] the modifications refer to a number of molecular mechanisms that regulate gene expression without changing DNA sequence[2]: (1) alteration of methylation status of DNA within CpG islands (the main human epigenetic modification)[3]; (2) covalent modification of histone tails; (3) gene regulation by micro-RNA (miRNA).

While early embryonic cells lack methylation (as it is not transmitted via the germline), methylation is essential for the development and regulation of gene expression and controls expression of oncofetal genes in postnatal life by imprinted genes and tissue-specific gene expression.[2] This perfect equilibrium in normal cells is transformed in cancer cells. DNA methylation is believed to contribute to cancer initiation and progression by gene inactivation. This can have important consequences if the inactivated genes are essential for the control of normal cell growth, differentiation, or apoptosis.[4] The mechanisms that regulate normal and aberrant methylation are neither fully understood nor are the mechanisms of methylation that interfere with transcription.[4]

The mismatch DNA repair (MMR) system is composed of a few well-conserved proteins. The essential components of MMR system, MutS, MutL, MutH and Uvr, were identified in *Escherichia coli*. In addition, all eukaryotic organisms, including humans, have MutS homologs and MutL homologs. The MLH1 and MSH2 genes provide instructions for making a protein that plays an essential role in DNA repair. These proteins fix mistakes that are made when DNA is copied (DNA replication) in preparation for cell division. The MLH1 protein joins with another protein, the PMS2, to form an active protein complex. This protein complex coordinates the activities of other proteins that repair errors during DNA replication. The repairs occur by removing the section of DNA and replacing it by a correct DNA sequence. The MSH2 protein joins with one of the two other proteins, the MSH6 protein or the MSH3 protein, to form an active protein complex. This active protein complex identifies places on the DNA where mistakes have been made during DNA replication.

The prognosis of lung cancer is very limited by the difficulties of diagnosing early stage disease amenable to surgery. Only 10% of cases can benefit from local treatment with long-term survival. Despite much progress in the treatment and detection methods of lung carcinoma, the prognosis remains poor. This situation is mainly the result of metastases which are present in more than two-thirds of patients at the time of diagnosis.[5,6]

The objectives of our study were to characterize the expression of DNA repair proteins MLH1 and MSH2 in tumour tissue, precursor lesions, respiratory epithelia and parenchyma of 80 clinically well-characterized NSCLC patients and also to study the methylation status of two DNA repair genes – *MLH1* and *MSH2*, to correlate between the methylation status of the promoters of *MLH1* and *MSH2*, and their respective protein expression and clinicopathological parameters.