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Editorial Comment

Tumour-associated hypermethylation: silencing E-cadherin expression enhances invasion and metastasis

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Cellular transformation is characterised by alterations in the organisation of the cytoskeleton, decreased cell-to-cell contacts, and increased cell proliferation. Thus, disruption of normal cell-cell adhesion in transformed cells contributes directly to tumour cells' enhanced migration and proliferation, leading to invasion and metastasis. One of the most studied molecules involved in cell-cell adhesion is E-cadherin, a 120 kDa transmembrane glycoprotein. The cytoplasmatic moiety of E-cadherin binds to β- and γ-catenin, which are linked to the cytoskeleton via α-catenin, while the extracellular moiety is a calcium-dependent receptor responsible for homophilic (E-cadherin/E-cadherin) interactions [1].

Consistent with its role in cell adhesion, germline- or somatic-inactivating mutations in the *E-cadherin* gene have been found in families with an inherited predisposition to gastric carcinomas [2–4] or in sporadic breast carcinomas and gastric carcinomas [5], respectively. However, a much larger fraction of cancers have reduced or absent E-cadherin RNA or protein expression, yet no mutations in the *E-cadherin* gene could be detected [6]. Several experimental observations suggest that other mechanisms might be involved in E-cadherin inactivation, such as promoter hypermethylation and changes in chromatin structure, as well as alterations of specific transcription factor pathways regulating *E-cadherin* gene expression. Indeed, a reduction in E-cadherin expression, which functions as an invasion suppressor in human cancers, is strongly related to invasiveness and metastasis *in vitro*.

DNA methylation in mammalian cells occurs at the 5-position of cytosine within the CpG dinucleotide [7]. This reaction is catalysed by the DNA methyltransferase (DNMT) enzymes. DNA methylation is the most common eukaryotic DNA modification and is one of the many epigenetic (alterations in gene expression without a change in nucleotide sequence) phenomena.

Eukaryotic genomes are not methylated uniformly, but contain methylated regions interspersed with unmethylated domains. The methylated regions are typical of the bulk chromatin (constitutive heterochromatin), while, in the rest of the genome, smaller regions of DNA, called CpG islands, overlap usually with gene promoters and contain an high frequency of unmethylated CpGs. Dense methylation of cytosine residues within these islands causes strong and heritable transcriptional silencing. Such silencing normally occurs almost solely at genes that are subject to genomic imprinting or to X chromosome inactivation. Intriguingly, methylation patterns become significantly altered during the carcinogenic process, with an overall hypomethylation of the genome and hypermethylation of specific (promoter) regions that leads to a 'close' chromatin structure [7]

Until recently, it was not clear whether the alteration in the methylation state is causative for carcinogenesis or is a by-product of the cancer state. We have recently reported that the leukaemia-promoting PML-RAR fusion protein induces gene hypermethylation and silencing by recruiting DNA methyltransferases to target promoters [8]. Importantly, this hypermethylation was found to contribute to the leukaemogenic potential of PML-RAR. Thus, alterations in methylation contribute directly to the carcinogenic potential of the cells. More in general, DNA methylation often causes the downregulation of tumour suppressor genes (such as *pRb*, *p15 INK4a*, *p16INK4a*) in cancer cells by changing chromatin structure, thereby making the DNA inaccessible for transcription factors and RNA polymerase II [9]. Aberrant *E-cadherin* promoter hypermethylation falls into this category. This notion is now reinforced by

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two studies presented in this issue of *European Journal of Cancer* [10,11].

Tsao and coworkers [10] report that E-cadherin expression was downregulated in 50% of nasopharyngeal carcinoma cell lines. Low levels of E-cadherin protein expression correlated with low levels of *E-cadherin* mRNAs, suggesting a defect at the transcriptional level. Indeed, analysis of the promoter region revealed a dramatic increase of CpGs methylation, establishing a strong link between *E-cadherin* silencing and the epigenetic state of its promoter. Furthermore, the authors also investigated the levels of promoter methylation in the primary nasopharyngeal carcinoma samples, obtaining a striking correlation with the previous observations.

Similar data on *E-cadherin* hypermethylation in cervical cancer cells have been offered in the accompanying study [11].

Chen and coworkers [11] reported *E-cadherin* promoter hypermethylation in 60% of cervical cancer cell lines and 40% of primary cervical cancers. These data complement a previous report [12] showing downregulation of E-cadherin protein expression in cervical cancer. Moreover, *DNMT1* mRNA and activity was increased in the cervical cancer tissues if compared with normal counterparts. An interesting aspect of this study is that inhibition of DNMT1 expression using antisense oligonucleotides reduced *E-cadherin* promoter methylation and restored its expression. These reports contribute to the characterisation of *E-cadherin* promoter hypermethylation in several tumour samples, such as primary gastric carcinoma, thyroid carcinoma, oral squamous cell carcinoma, hepatocellular carcinoma, prostate and breast carcinomas.

The picture that emerges from the analysis of all of these studies suggests that promoter hypermethylation is the main mechanism involved in promoter silencing of *E-cadherin* in those tumours, although not the only one. The data presented in the two papers confirm that loss of heterozygosity (LOH) and/or point mutation (or even other mechanisms) also contributes to the downregulation of E-cadherin. The mechanistic question remains as to whether aberrant promoter methylation in those tumours is a causal and not consequent to malignant transformation. Future studies, probably investigating the status of *E-cadherin* promoter methylation at different stages (e.g. premalignant versus malignant lesions) might help in addressing this question.

As promoter methylation is reversible, it may provide an attractive target for the development of new anticancer therapies. The interesting aspect that emerges from the studies on E-cadherin is that the epigenetic alteration does not occur in the same tumour cells as those carrying genetic lesions (such as deletions and point mutations) since inhibition of DNMT activity (by antisense oligos or by demethylating agent 5-Aza CdR) leads to gene re-expression. Indeed, the clinical benefits of DNMTs inhibition and their implications for re-differentiation therapy are currently being investigated in several locations for several types of tumours. Restoration of the correct E-cadherin expression can directly reduce cell motility and invasiveness, and thus the formation of metastases.

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