

Commentary

E-cadherin and loss of heterozygosity at chromosome 16 in breast carcinogenesis: different genetic pathways in ductal and lobular breast cancer?

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Abstract

Loss of heterozygosity at the long arm of chromosome 16 is one of the most frequent genetic events in breast cancer. In the search for tumour suppressor genes that are the target of loss of heterozygosity at 16q, the E-cadherin gene *CDH1* was unveiled by the identification of truncating mutations in the retained copy. However, only lobular tumours showed E-cadherin mutations. Whereas investigations are still devoted to finding the target genes in the more frequent ductal breast cancers, other studies suspect the E-cadherin gene to also be the target in this tumour type. The present article discusses the plausibility of those two lines of thought.

Keywords: hereditary cancer, loss of heterozygosity, mutation, tumour progression, tumour suppressor genes

Introduction

The homotypic cellular adhesion molecule E-cadherin is one of the most vital components in the cell. It is implicated as a key player in different cellular processes including development, morphology, polarity, migration and tissue integrity [1]. E-cadherin is a glycoprotein with an extracellular domain that interacts with E-cadherin molecules on adjacent cells, thereby establishing adhesion between epithelial cells. The intracellular domain is associated with a complex of proteins called catenins, which anchor E-cadherin to the actin cytoskeleton.

In various carcinomas, plasma membrane associated E-cadherin protein expression is decreased or even absent. There is transcriptional regulation of this molecule for which a number of factors have been implicated, including promotor hypermethylation [2,3], and several proteins that regulate E-cadherin transcription, especially Snail-1 [4], SIP-1 [5] and integrin-linked kinase [6]. Muta-

tional inactivation of the E-cadherin gene *CDH1* has been reported in diffuse gastric cancer and lobular breast cancer [7]. Both these tumour types have a characteristic diffuse growth pattern with loss of cellular coherence that is in accordance with the adhesion function of the absent E-cadherin protein.

The wild type *CDH1* allele is missing in most lobular tumours due to loss of heterozygosity (LOH) at chromosome 16q [8], thereby presenting a classical example of Knudson's two-hit hypothesis on the inactivation of tumour suppressor genes. The more frequent ductal breast carcinomas also show frequent LOH at 16q; however, these tumours do not have mutational inactivation of the retained *CDH1* allele [9]. Given the importance and widespread involvement of E-cadherin in tumorigenic processes, it is tempting to assume that a decrease in E-cadherin activity in ductal carcinomas is selected for and is reflected by LOH at 16q. Indeed, haploinsufficiency has now been

acknowledged as a true mechanism for tumour suppressor gene inactivation [10]. However, other genes at 16q could be targets of LOH in ductal tumours, justifying ongoing gene hunts.

LOH at 16q in ductal and lobular breast cancer

LOH at 16q is the second most frequent somatic genetic event in breast cancer. This event occurs in about 50% of all ductal carcinomas [11] and is slightly more frequent in lobular breast cancer [8]. To confirm E-cadherin as the target of LOH in ductal carcinoma, it is important to distinguish physical loss and mitotic recombination [12,13]. Only the first LOH event could theoretically lead to haploinsufficiency of *CDH1*. This seems unlikely, however, since there are so many complex mechanisms for regulation of *CDH1* transcription that are often part of feedback loops [14]. Furthermore, our observations (unpublished data) on LOH at 16q in breast cancer indicate that both mechanisms for LOH are operative.

E-cadherin protein expression in ductal and lobular breast cancer

The complete absence of E-cadherin plasma membrane associated protein expression as detected by immunohistochemistry is so unambiguous that pathologists use this immunostaining to confirm their diagnosis of lobular breast cancer. We have stained a series of 86 breast carcinomas with known E-cadherin mutation status for E-cadherin protein expression [15]. Complete loss of protein was found in all lobular tumours with mutational inactivation of E-cadherin ($n = 21$). In addition, we were unable to detect E-cadherin in 11 lobular tumours in which no mutation had been identified. This is probably due to insensitivity of the mutation detection method or because other mechanisms of inactivation (e.g. methylation) were active. Remarkably, six cases of lobular breast cancer without a detectable E-cadherin mutation were positive for E-cadherin immunostaining. Of the 48 ductal breast cancers tested, 37% showed a decrease in but never a complete absence of E-cadherin protein expression. If E-cadherin was the target of LOH at 16q in ductal breast cancer, one would expect a strong association between LOH at 16q and decreased E-cadherin expression. However, the percentage of LOH at 16q in tumours with and without E-cadherin decrease was equal. It therefore seems unlikely that LOH at 16q is associated with a decrease in E-cadherin expression in ductal breast cancer. Also, in ductal carcinoma *in situ* (DCIS), there is no association between LOH at 16q and a decrease in E-cadherin expression. This can be derived from our combined data on LOH and E-cadherin immunohistochemistry, which was available for 62 cases of pure DCIS [16,17].

The adhesion function of E-cadherin is strongly indicative of a function in invasion suppression and this has indeed

been shown *in vitro* [18] and in mouse models [19]. Investigation in primary tumours showed that this function translates well into metastatic potential [20]. There is no significant correlation between LOH at 16q in breast cancer and metastatic potential [11], however, which further contradicts an association between E-cadherin and LOH at 16q.

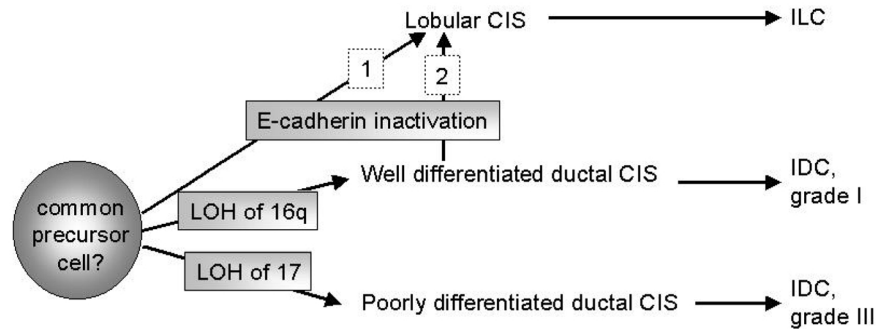
A breast cancer progression model

The invasion suppressor function of E-cadherin is very obvious, but not in concordance with our earlier finding [16] that mutational inactivation of both *CDH1* alleles through LOH and truncating mutations occurs in the preinvasive stage in lobular carcinoma *in situ* (LCIS), a tumour stage that involves proliferation but not dissemination. We showed that the same mutation and LOH at 16q was present in the invasive tumour and the adjacent LCIS [16]. E-cadherin may thus play a role in invasive capacity, but more data suggest other signalling mechanisms may be involved in earlier tumorigenic processes, especially cellular proliferation. Indeed, E-cadherin is implicated in several signalling pathways: Wnt, Rho/Rac and p27^{Kip1}, which are involved in transcriptional activation, actin cytoskeleton reorganisation and contact inhibition, respectively [14].

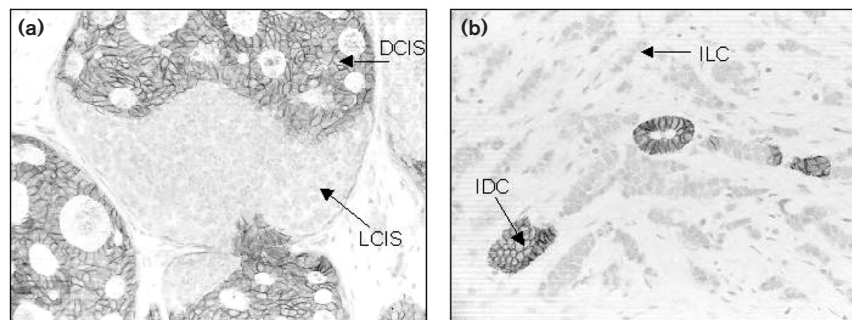
LOH at 16q also occurs in the preinvasive stage, predominantly in grade I DCIS and in LCIS [17,21]. These observations and data in the literature led to a progression model of breast cancer (Fig. 1) based on early genetic alterations [17]. A similar multistep model for breast carcinogenesis has been proposed by Lakhani [22]. The model by Vos *et al.* [17] is less well defined than the Vogelstein model for colorectal cancer, because the latter is based on specific tumour stages and gene mutations. Similar stages are not defined for breast cancer and most of the genes involved are not yet identified. However, somatic genetic alterations in DCIS, LCIS and invasive carcinoma indicate that breast cancer progression is also based on the accumulation of genetic alterations. LOH at 16q in grade I DCIS and LCIS led us to suggest that grade I DCIS may be a precursor for LCIS. Loss of E-cadherin further determines the histological fate of the tumour. Loss of chromosome 16q in grade I ductal and lobular breast cancer also lead Roylance *et al.* to speculate that these apparently morphological different tumours have a common molecular origin [23]. The observation of mixed populations of tumour cells, LCIS adjacent to DCIS, infiltrating lobular carcinoma and infiltrating ductal carcinoma (Fig. 2) supports this model, as well as investigations by Buerger *et al.* [24] on comparative genomic hybridisation of different tumour populations within the same lesion.

E-cadherin germline mutations and lack of LOH in gastric cancer

The identification of somatic E-cadherin mutations in breast and gastric cancer received less attention than

Figure 1

Model of breast cancer progression. Grade II tumours are omitted for simplification. Two possible pathways may result in lobular carcinoma: 1, direct; or 2, via well-differentiated ductal carcinoma *in situ* (CIS). IDC, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma; LOH, loss of heterozygosity.

Figure 2

E-cadherin staining of mixed populations of (a) ductal carcinoma *in situ* (DCIS) and lobular carcinoma *in situ* (LCIS), and (b) infiltrating ductal carcinoma (IDC) and infiltrating lobular carcinoma (ILC). The E-cadherin staining was performed with antibody HECD-1 and distinguishes ductal from lobular tumour cells. Courtesy of Dr CBJ Vos.

the report on two Maori families with diffuse gastric cancer attributed to germline transmission of truncating mutations in *CDH1* [25]. Although lobular breast cancer was expected, none was registered in these Maori families or in others reported on later [26]. Examination of *CDH1* in 65 patients with LCIS revealed no germline mutations [27]. Loss of one *CDH1* allele apparently gives an increased risk only for gastric cancer, both hereditary and sporadic.

Remarkably, at the level of LOH, there is also a substantial difference between breast and gastric tumours. In diffuse gastric tumours, the wild type *CDH1* allele is inactivated not by LOH at 16q, but by promoter methylation [28]. This marked difference in general genetic mechanism may reflect a difference in the role of the tumour suppressor gene. Whereas the loss of E-cadherin is a rate-limiting factor in gastric cancer, in breast cancer it probably plays a role in a later stage and it determines the histological subtype.

Conclusions

LOH at chromosome arm 16q in breast cancer is a frequent event, occurring in at least 50% of breast cancer cases. In lobular breast cancer, a histological minority comprising 5–10% of all breast cancers, the E-cadherin gene is the target of this somatic genetic event. In ductal breast cancer it is unlikely that *CDH1* is the target tumour suppressor gene, and other genes therefore remain to be identified. Classical LOH mapping efforts have not been successful in the identification of these target genes at chromosome 16, or other genes in other tumour types, and we therefore need to apply different high-throughput screening methods to identify these remaining genes.

The E-cadherin gene has many different functions, even in carcinogenesis, given its involvement in early lesions and metastasis, hereditary and sporadic tumours, and numerous different tumour types. To elucidate whether this remarkable diversity indicates true separate activities or is a reflection of this protein's central role in cellular

processes will be a challenging task for cellular biologists, geneticists and oncology researchers together.

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