

Commentary

The E-cadherin/catenin complex: an important gatekeeper in breast cancer tumorigenesis and malignant progression

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(Print ISSN 1465-5411; Online ISSN 1465-542X)**Abstract**

E-cadherin is a cell–cell adhesion protein fulfilling a prominent role in epithelial differentiation. Data from model systems suggest that E-cadherin is a potent invasion/tumor suppressor of breast cancer. Consistent with this role in breast cancer progression, partial or complete loss of E-cadherin expression has been found to correlate with poor prognosis in breast cancer patients. The E-cadherin gene (*CDH1*) is located on human chromosome 16q22.1, a region frequently affected with loss of heterozygosity in sporadic breast cancer. Invasive lobular breast carcinomas, which are typically completely E-cadherin-negative, often show inactivating mutations in combination with loss of heterozygosity of the wild-type *CDH1* allele. Mutations were found at early noninvasive stages, thus associating E-cadherin mutations with loss of cell growth control and defining *CDH1* as the tumor suppressor for the lobular breast cancer subtype. Ductal breast cancers in general show heterogeneous loss of E-cadherin expression, associated with epigenetic transcriptional downregulation. It is proposed that the microenvironment at the invasive front is transiently downregulating E-cadherin transcription. This can be associated with induction of nonepithelial cadherins.

Keywords: E-cadherin, methylation, mutation, transcriptional repression, tumor suppression**Introduction**

Malignant breast cancer is a disease arising in the ductal and lobular epithelium of the mammary gland. Intercellular interactions are critical for the dynamic differentiation processes activated periodically throughout life in normal breast epithelium, as well as for the induction and maintenance of differentiated tissues in adults. In the past few years there has been increasing interest in E-cadherin-mediated cell–cell adhesion and cell–extracellular matrix adhesion as prime mediators of epithelial differentiation.

E-cadherin is a glycoprotein with a large extracellular domain comprising five cadherin-motif subdomains, a

single-pass transmembrane segment and a short conserved cytoplasmic domain, which interacts with several proteins collectively termed catenins. Several of these catenins belong to the Armadillo protein family, whereas α -catenins are vinculin-related molecules. The central armadillo domain of either β -catenin or plakoglobin (γ -catenin) interacts directly with a carboxy-terminal cytoplasmic domain of the E-cadherin protein. The same armadillo molecules also interact with α -catenin, and the latter is directly and indirectly linked to filamentous actin. Another armadillo catenin, p120^{ctn}, interacts with a more membrane-proximal cytoplasmic domain of cadherins. The 120^{ctn} protein is involved in strengthening cadherin-

containing adhesion junctions [1]. Whereas β -catenin is a proto-oncogene by virtue of its important role in the Wnt signaling pathway [2], E-cadherin exerts a potent invasion-suppressing role in tumor cell lines and *in vivo* tumor model systems [3–6]. Forced expression of E-cadherin decreased proliferation of different mammary carcinoma cell lines [4,7]. Precancerous hyperproliferation and sustained activation of the Ras-MAPK cascade was recently found in skin with tissue-specific ablation of α -catenin [8].

Disturbance of E-cadherin expression in breast cancer

Breast E-cadherin is expressed in normal adults in luminal epithelial cells, whereas expression of P-cadherin is confined to myoepithelial cells [9,10]. Temporary downregulation of E-cadherin was found in budding lobules invading the stroma of breast tissue [11]. Changes in the normal expression pattern of the E-cadherin/catenin complex have been found in various human cancers. In breast cancer, generally speaking, partial or total loss of E-cadherin expression correlates with loss of differentiation characteristics, acquisition of invasiveness, increased tumor grade, metastatic behavior and poor prognoses [12–15]. Taking into account the two major histological subtypes of breast cancer, however, different modes of E-cadherin expression modulation have been found. While infiltrating ductal breast cancers mostly show no or only heterogeneously reduced E-cadherin expression, infiltrative lobular breast carcinomas (ILC) are, in most cases (85%), completely E-cadherin-negative [9,16–19]. A significantly lower ratio of E-cadherin-negative versus E-cadherin-positive ILC samples has been reported by other workers [19,20]. This discrepancy could be partly owing to diagnostic variation as applied to lobular carcinomas [21].

In addition to loss of E-cadherin expression in ILC, simultaneous loss of α -catenin expression and β -catenin expression has been observed [22]. Interestingly, in a minority (15%) of ILC cases, expression of E-cadherin and catenins is maintained. In these cases, however, E-cadherin expression is atypical because it is nonpolarized (i.e. tumor cells are stained all over their surface), pointing toward dysfunction of normal cell–cell adhesion properties [19,20,22]. Intriguing is the finding that, although primary ductal and lobular breast cancers can show partial or complete loss of E-cadherin expression, their derivative metastases may exhibit strong E-cadherin expression [23,24]. This suggests that transient E-cadherin downregulating mechanisms might be involved in malignant cancers without irreversible mutations of the E-cadherin gene. The observed switches of cadherin expression in breast cancer cell lines and tumors are also important [25,26]. High-grade ductal breast lesions with reduced E-cadherin expression may show abnormal P-cadherin expression in luminal cells. Moreover, reduced E-cadherin expression in breast cancer cells is often associated with

inappropriate expression of N-cadherin and cadherin-11, which are typically expressed in mesenchymal cells. Forced expression of N-cadherin in E-cadherin-positive breast cancer cells correlates with invasion and motility, suggesting that N-cadherin plays an important role in promoting these malignant features [26].

Irreversible inactivation of E-cadherin in breast cancer

The efforts to allelotype breast cancer showed concurrent loss of heterozygosity (LOH) at multiple chromosomal sites, with LOH at 16q being one of the most common events (52.3%) in sporadic breast cancer [27]. This points to a significant role of the genes in this chromosomal region to generate sporadic breast cancer. The E-cadherin gene (*CDH1*) maps to the human chromosome 16q22.1 [28]. Somatic mutations in *CDH1* were found in about 56% of lobular breast tumors, generally (>90%) in combination with loss of the wild-type allele, while no mutations were found in ductal primary breast carcinomas [28,29]. Most of these somatic mutations result in premature stop codons as a consequence of insertions, deletions and nonsense mutations. As the majority of these frameshift and nonsense mutations is predicted to generate secreted E-cadherin fragments, the functionality of this major cell–cell adhesion protein is lost. Other cancer-confined E-cadherin mutations also result in crippled proteins. The distinctive invasive growth pattern, which is typical for lobular breast cancers, is fully compatible with this functional inactivation. The finding that loss of E-cadherin immunoreactivity and corresponding mutations are already present in early noninvasive lobular carcinoma *in situ* (LCIS) lesions is intriguing [18]. This suggests a genuine tumor suppressor role for E-cadherin during sporadic breast cancer development, in addition to the previously described role as an invasion suppressor. Experimental evidence for an effective role in control of cell proliferation comes from *in vitro* and *in vivo* experiments with E-cadherin-negative breast cancer cells. Forced E-cadherin expression in these cells resulted in significant growth inhibition both in cell culture and as tumors in mice [4]. This diminished growth capacity could be associated with elevated expression of the cyclin-dependent kinase inhibitor p27^{KIP1} [7].

The molecular basis of mixed types of ductal/lobular breast cancers remains, however, enigmatic. Such mixed breast cancer type occurs in both advanced and *in situ* stages. This might indicate that the lobular component in these particular breast tumors could originate from ductal carcinomas (*in situ* or infiltrative variants) through E-cadherin inactivation. This would be fully compatible with earlier findings in mixed gastric carcinomas where E-cadherin mutations were exclusively observed in the diffuse component of the tumors [30].

Complete loss of E-cadherin expression may be expected to result in increased levels of free cytoplasmic or nuclear β -catenin. The nuclear forms of β -catenin accumulate in colorectal cancers due to mutations in the Wnt signaling pathway, including truncating adenomatous polyposis coli mutation and stabilizing β -catenin mutations [2]. The outcome is interaction of β -catenin with transcription factors of the lymphoid enhancer factor–T-cell factor family in the nucleus and modulation of gene expression. The concomitant loss of α -catenin expression and β -catenin expression in ILCs with mutated *CDH1* gene [22], however, rules out the possible activation of Wnt signaling through accumulating free cytoplasmic or nuclear β -catenin in these particular breast cancers. Indeed, it has recently become clear that cell lines of either lobular or ductal origin with loss of E-cadherin expression do not show enhanced Wnt signaling [31]. Nonetheless, expression of a stabilized, transcriptionally active form of β -catenin in the mammary gland of transgenic mice affected normal gland differentiation and induced readily multiple aggressive adenocarcinomas with overexpression of both c-Myc and cyclin D1 [32]. Surprisingly, for ILCs with a defective E-cadherin/catenin complex but lacking detectable E-cadherin mutations, no evidence has so far been found for mutational inactivation of α -catenin or β -catenin [33]. In conflict with mutational data from primary tumors is also the report that 20% (3/15) of breast cancer cell lines of ductal origin carry inactivating E-cadherin mutations in combination with LOH [31]. The reason for this discrepancy is presently unclear: it could be a reflection of deviating histopathological diagnosis, or it may be owing to tumor heterogeneity in primary ductal carcinomas with a hardly detectable minority of cells of the ILC type with E-cadherin mutations. Moreover, the existence of ductal breast cancer cell lines with loss of functional E-cadherin may be the result of *in vitro* clonal selection due to growth advantage provided by inactivation of the E-cadherin gene.

The often-occurring inactivation of the *CDH1* gene in sporadic ILC of the breast as well as the high frequency of multicentric and bilateral ILC or LCIS strongly suggested E-cadherin as a good candidate tumor suppressor gene for a fraction of the hereditary breast cancers [34,35]. E-cadherin germ-line mutations have been identified in a number of families with inherited predisposition to diffuse gastric carcinomas [36,37]. A subset of patients with this inherited cancer syndrome, also termed hereditary diffuse gastric cancer syndrome, exhibit other cancers such as breast and colorectal cancer. Constitutional *CDH1* mutations in a single family have so far been associated with the development of metachronous diffuse gastric cancer and breast cancer of the lobular subtype [38]. The reason for the apparent predominance of gastric cancer in these families is so far unknown; it may be the consequence of a differential inactivation efficiency of the second allele by genetic or epigenetic mechanisms [39]. Patients

diagnosed with LCIS do consistently show LOH for 16q22.1 and loss of E-cadherin expression, and somatic mutations in the *CDH1* gene of LCIS cells were indeed reported [18,22]. Nevertheless, no constitutional mutations could be identified in a large series of LCIS [40].

Reduced transcription of E-cadherin in breast cancer

For most of the primary breast cancers and cell lines of the ductal histotype, no E-cadherin mutations could be identified despite the fact that these tumors often show strikingly reduced E-cadherin gene and protein expression. Possible mechanisms to explain this reduced expression include chromatin rearrangements, hypermethylation and alterations in *trans*-factor binding [41,42]. Hypermethylation of the *CDH1* promoter and the overlapping 5' CpG island has been demonstrated to correlate with loss of E-cadherin expression at the transcriptional level for various breast cancer cell lines and primary ductal breast cancers [43]. Moreover, several infiltrative lobular cancers were recently reported to carry methylated *CDH1* promoter sequences [20]. This might serve as a second gene inactivation event, in combination with either LOH or somatic *CDH1* mutations, although biallelic methylation was also assumed to occur. Treatment of two breast cancer cell lines with the DNA methylation inhibitor 5-aza-2'-deoxycytidine resulted in slight upregulation of E-cadherin mRNA and protein levels [43]. Interestingly, heterogeneous methylation of this 5' CpG island has been reported to markedly increase during malignant progression from ductal carcinoma *in situ* to metastatic lesions [44]. These epigenetic changes appear to be dynamic as they can be mimicked *in vitro* depending on microenvironmental conditions favoring either homotypic cell adhesion (growth as spheroids) or *in vitro* invasion [45].

It is not yet clear, however, whether the direct involvement of hypermethylation as a predominant mechanism in suppressing E-cadherin gene expression can be extrapolated to most breast cancers showing a methylated gene promoter. It is indeed intriguing that E-cadherin expression could not be restored in somatic cell hybrids resulting from fusions between E-cadherin-positive cell lines and cell lines with a methylated inactive E-cadherin promoter [46]. Besides this dominant repression, the inability to reactivate E-cadherin expression by 5-aza-2'-deoxycytidine treatment has been seen, which indicates that loss of E-cadherin mRNA expression is not only attributable to hypermethylation [47]. Support for a *trans*-acting repression mechanism has recently been found by identification of the transcription factor Snail, binding directly to E2 boxes in the E-cadherin promoter and potently repressing its transcription [48,49]. Snail is highly expressed in E-cadherin-negative breast cancer cell lines, including those with methylated 5' CpG islands. In this context, it is interesting to mention that high integrin-linked kinase

expression in mammary epithelial cells induced an epithelial→mesenchymal transition, which was associated with loss of E-cadherin expression [50]. It recently became apparent that integrin-linked kinase activates the Snail promoter in colorectal cancer cell lines with adenomatous polyposis coli mutations [51]. Moreover, E-cadherin gene transcription has been reported to be inhibited in breast cancer cells by overexpression of *erbB2*, a proto-oncogene frequently overexpressed in breast cancers [52]. Transforming growth factor- β , which plays an important inhibitory role in lobuloalveolar development on overexpression *in vivo* in mammary gland [53], is also able to repress E-cadherin transcription in mammary gland cells [54,55]. It is therefore most interesting that the two-handed E2-box binding zinc finger protein SIP1 (ZEB2), initially isolated on the basis of its interaction with transforming growth factor- β -regulated Smad proteins, can downregulate E-cadherin and induce invasiveness [56]. Various E-cadherin-negative breast and colon cancer cell lines express SIP1, including those with a methylated E-cadherin promoter. It may therefore be worthwhile to seek Snail and SIP1-inducing factors in the microenvironment of invasive parts of malignant breast tumors.

Conclusions

Today, it is doubtless that inactivation of E-cadherin has an important role in the development of part of the sporadic breast cancers. The high incidence of complete and irreversible inactivation of E-cadherin in infiltrative lobular breast cancer evidences the role of E-cadherin as a genuine tumor suppressor in this specific histological subclass of sporadic breast cancers. This is further supported by the finding that E-cadherin is already inactivated in early noninvasive LCIS, which contradicts a model with a restricted role for E-cadherin as only an invasion suppressor. Heterogeneous loss of E-cadherin expression is generally observed in ductal breast cancers. This negative regulation seems to be reversible at the transcriptional level, allowing re-expression at the secondary metastatic tumor site. As the underlying mechanisms and relevant key molecules become progressively identified, this opens the possibility to develop anti-tumor and anti-invasion strategies aimed at functional upregulation of E-cadherin in breast cancers.

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