Commentary

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

Geert Berx and Frans Van Roy

Molecular Cell Biology Unit, Department of Molecular Biology, Flanders Interuniversity Institute for Biotechnology (VIB) – Ghent University, Ghent, Belgium

Correspondence: Prof Frans Van Roy, Molecular Cell Biology Unit, Department of Molecular Biology, VIB – University of Ghent, Ledeganckstraat 35, B-9000 Ghent, Belgium. Tel: +32 9 264 5017; fax: +32 9 264 5348; e-mail: f.vanroy@dmb.rug.ac.be

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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-

ILC = infiltrative lobular breast carcinomas; LCIS = lobular carcinoma *in situ*; LOH = loss of heterozygosity.

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]. Somatically acquired 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 *erb*B2, 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.

References

1. Thoreson MA, Anastasiadis PZ, Daniel JM, Ireton RC, Wheelock MJ, Johnson KR, Hummingbird DK, Reynolds AB: **Selective uncoupling of p120ctn from E-cadherin disrupts strong adhesion**. *J Cell Biol* 2000, **148**:189-201.
2. Behrens J: **Cadherins and catenins: Role in signal transduction and tumor progression**. *Cancer Metastasis Rev* 1999, **18**:15-30.
3. Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Löchner D, Birchmeier W: **E-cadherin-mediated cell–cell adhesion prevents invasiveness of human carcinoma cells**. *J Cell Biol* 1991, **113**:173-185.
4. Meiners S, Brinkmann V, Naundorf H, Birchmeier W: **Role of morphogenetic factors in metastasis of mammary carcinoma cells**. *Oncogene* 1998, **16**:9-20.
5. Perl AK, Wilgenbus P, Dahl U, Semb H, Christofori G: **A causal role for E-cadherin in the transition from adenoma to carcinoma**. *Nature (London)* 1998, **392**:190-193.
6. Vleminckx K, Vakaet Jr L, Mareel M, Fiers W, van Roy F: **Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role**. *Cell* 1991, **66**:107-119.
7. St Croix B, Sheehan C, Rak JW, Florenes VA, Slingerland JM, Kerbel RS: **E-Cadherin-dependent growth suppression is mediated by the cyclin-dependent kinase inhibitor p27^{KIP1}**. *J Cell Biol* 1998, **142**:557-571.
8. Vasioukhin V, Bauer C, Degenstein L, Wise B, Fuchs E: **Hyperproliferation and defects in epithelial polarity upon conditional ablation of alpha-catenin in skin**. *Cell* 2001, **104**:605-617.
9. Rasbridge SA, Gillett CE, Sampson SA, Walsh FS, Millis RR: **Epithelial (E-) and placental (P-) cadherin cell adhesion molecule expression in breast carcinoma**. *J Pathol* 1993, **169**:245-250.
10. Palacios J, Benito N, Pizarro A, Suarez A, Espada J, Cano A, Gamallo C: **Anomalous expression of P-cadherin in breast carcinoma: correlation with E-cadherin expression and pathological features**. *Am J Pathol* 1995, **146**:605-612.
11. Daniels C, Strickland P, Friedmann Y: **Expression and functional role of E- and P-cadherin in mouse mammary ductal morphogenesis and growth**. *Dev Biol* 1995, **169**:511-519.
12. Heimann R, Lan FS, McBride R, Hellman S: **Separating favorable from unfavorable prognostic markers in breast cancer: the role of E-cadherin**. *Cancer Res* 2000, **60**:298-304.
13. Hunt NCA, DouglasJones AG, Jasani B, Morgan JM, Pignatelli M: **Loss of E-cadherin expression associated with lymph node metastases in small breast carcinomas**. *Virchows Arch Int J Pathol* 1997, **430**:285-289.
14. Oka H, Shiozaki H, Kobayashi K, Inoue M, Tahara H, Kobayashi T, Takatsuka Y, Matsuyoshi N, Hirano S, Takeichi M, Mori T: **Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis**. *Cancer Res* 1993, **53**:1696-1701.
15. Siitonen SM, Kononen JT, Helin HJ, Rantala IS, Holli KA, Isola JJ: **Reduced E-cadherin expression is associated with invasiveness and unfavorable prognosis in breast cancer**. *Am J Clin Pathol* 1996, **105**:394-402.
16. Gamallo C, Palacios J, Suarez A, Pizarro A, Navarro P, Quintanilla M, Cano A: **Correlation of E-cadherin expression with differentiation grade and histological type in breast carcinoma**. *Am J Pathol* 1993, **142**:987-993.
17. Moll R, Mitze M, Frixen UH, Birchmeier W: **Differential loss of E-cadherin expression in infiltrating ductal and lobular breast carcinomas**. *Am J Pathol* 1993, **143**:1731-1742.
18. Vos CBJ, Cleton-Jansen A-M, Berx G, de Leeuw WJF, ter Haar NT, van Roy F, Cornelisse CJ, Peterse JL, van de Vijver MJ: **E-cadherin inactivation in lobular carcinoma in situ of the breast: an early event in tumorigenesis**. *Br J Cancer* 1997, **76**:1131-1133.
19. Huiping C, Sigurgeirsdottir JR, Jonasson JG, Eiriksdottir G, Johannsdottir JT, Egilsson V, Ingvarsson S: **Chromosome alterations and E-cadherin gene mutations in human lobular breast cancer**. *Br J Cancer* 1999, **81**:1103-1110.
20. Droufakou S, Deshmane V, Roylance R, Hanby A, Tomlinson I, Hart IR: **Multiple ways of silencing E-cadherin gene expression in lobular carcinoma of the breast**. *Int J Cancer* 2001, **92**:404-408.
21. Cserni G: **Reproducibility of a diagnosis of invasive lobular carcinoma**. *J Surg Oncol* 1999, **70**:217-221.
22. de Leeuw WJF, Berx G, Vos CBJ, Peterse JL, van de Vijver MJ, Litvinov S, van Roy FM, Cornelisse CJ, Cleton-Jansen A-M: **Simultaneous loss of E-cadherin and catenins in invasive lobular breast cancer and lobular carcinoma in situ**. *J Pathol* 1997, **183**:404-411.
23. Bukholm IK, Nesland JM, Borresen-Dale AL: **Re-expression of E-cadherin, alpha-catenin and beta-catenin, but not of gamma-catenin, in metastatic tissue from breast cancer patients**. *J Pathol* 2000, **190**:15-19.
24. Mareel MM, Behrens J, Birchmeier W, De Bruyne GK, Vleminckx K, Hoogewijs A, Fiers WC, van Roy FM: **Down-regulation of E-cadherin expression in Madin Darby canine kidney (MDCK) cells inside tumors of nude mice**. *Int J Cancer* 1991, **47**:922-928.

25. Hazan RB, Kang L, Whooley BP, Borgen PI: **N-cadherin promotes adhesion between invasive breast cancer cells and the stroma**. *Cell Adhes Commun* 1997, **4**:399–411.
26. Nieman MT, Prudoff RS, Johnson KR, Wheelock MJ: **N-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression**. *J Cell Biol* 1999, **147**:631-643.
27. Cleton-Jansen A-M, Moerland EW, Kuipers-Dijkshoorn NJ, Callen DF, Sutherland GR, Hansen B, Devilee P, Cornelisse CJ: **At least two different regions are involved in allelic imbalance on chromosome arm 16q in breast cancer**. *Gene Chromosome Cancer* 1994, **9**:101-107.
28. Berx G, Cleton-Jansen A-M, Nollet F, de Leeuw WJF, van de Vijver MJ, Cornelisse C, van Roy F: **E-cadherin is a tumor/invasion suppressor gene mutated in human lobular breast cancers**. *EMBO J* 1995, **14**:6107-6115.
29. Berx G, Cleton-Jansen A-M, Strumane K, de Leeuw WJF, Nollet F, van Roy FM, Cornelisse C: **E-cadherin is inactivated in a majority of invasive human lobular breast cancers by truncation mutations throughout its extracellular domain**. *Oncogene* 1996, **13**:1919-1925.
30. Machado JC, Soares P, Carneiro F, Rocha A, Beck S, Blin N, Berx G, Sobrinho-Simoes M: **E-cadherin gene mutations provide a genetic basis for the phenotypic divergence of mixed gastric carcinomas**. *Lab Invest* 1999, **79**:459-465.
31. van de Wetering M, Barker N, Harkes IC, van der Heyden M, Dijk NJ, Hollestelle A, Klijn JG, Clevers H, Schutte M: **Mutant E-cadherin breast cancer cells do not display constitutive Wnt signaling**. *Cancer Res* 2001, **61**:278-284.
32. Imbert A, Eelkema R, Jordan S, Feiner H, Cowin P: **DeltaN89beta-catenin induces precocious development, differentiation, and neoplasia in mammary gland**. *J Cell Biol* 2001, **153**:555-568.
33. Candidus S, Bischoff P, Becker KF, Hofler H: **No evidence for mutations in the alpha- and beta-catenin genes in human gastric and breast carcinomas**. *Cancer Res* 1996, **56**:49-52.
34. Rosen PP, Lesser ML, Senie RT, Duthie K: **Epidemiology of breast carcinoma IV: age and histologic tumor type**. *J Surg Oncol* 1982, **19**:44-51.
35. Claus EB, Risch N, Thompson WD, Carter D: **Relationship between breast histopathology and family history of breast cancer**. *Cancer* 1993, **71**:147-153.
36. Gayther SA, Gorringe KL, Ramus SJ, Huntsman D, Roviello F, Grehan N, Machado JE, Pinto E: **Identification of germ-line E-cadherin mutations in gastric cancer families of European origin**. *Cancer Res* 1998, **58**:4086-4089.
37. Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taite H, Scoular R, Miller A, Reeve AE: **E-cadherin germline mutations in familial gastric cancer**. *Nature (London)* 1998, **392**:402-405.
38. Keller G, Vogelsang H, Becker I, Hutter J, Ott K, Candidus S, Grundei T, Becker KF, Mueller J, Siewert JR, Hofler H: **Diffuse type gastric and lobular breast carcinoma in a familial gastric cancer patient with an E-cadherin germline mutation**. *Am J Pathol* 1999, **155**:337-342.
39. Grady WM, Willis J, Guilford PJ, Dunbier AK, Toro TT, Lynch H, Wiesner G, Ferguson K, Eng C, Park JG, Kim SJ, Markowitz S: **Methylation of the CDH1 promoter as the second genetic hit in hereditary diffuse gastric cancer**. *Nat Genet* 2000, **26**:16-17.
40. Rahman N, Stone JG, Coleman G, Gusterson B, Seal S, Marossy A, Lakhani SR, Ward A, Nash A, McKinna A, AHern R, Stratton MR, Houlston RS: **Lobular carcinoma in situ of the breast is not caused by constitutional mutations in the E-cadherin gene**. *Br J Cancer* 2000, **82**:568-570.
41. Yoshiura K, Kanai Y, Ochiai A, Shimoyama Y, Sugimura T, Hirohashi S: **Silencing of the E-cadherin invasion-suppressor gene by CpG methylation in human carcinomas**. *Proc Natl Acad Sci USA* 1995, **92**:7416-7419.
42. Hennig G, Lowrick O, Birchmeier W, Behrens J: **Mechanisms identified in the transcriptional control of epithelial gene expression**. *J Biol Chem* 1996, **271**:595-602.
43. Graff JR, Herman JG, Lapidus RG, Chopra H, Xu R, Jarrard DF, Isaacs WB, Pitha PM, Davidson NE, Baylin SB: **E-cadherin expression is silenced by DNA hypermethylation in human breast and prostate carcinomas**. *Cancer Res* 1995, **55**:5195-5199.
44. Nass SJ, Herman JG, Gabrielson E, Iversen PW, Parl FF, Davidson NE, Graff JR: **Aberrant methylation of the estrogen receptor and E-cadherin 5′ CpG islands increases with malignant progression in human breast cancer**. *Cancer Res* 2000, **60**:4346-4348.
45. Graff JR, Gabrielson E, Fujii H, Baylin SB, Herman JG: **Methylation patterns of the E-cadherin 5′ CpG island are unstable and reflect the dynamic, heterogeneous loss of E-cadherin expression during metastatic progression**. *J Biol Chem* 2000, **275**:2727-2732.
46. Hajra KM, Ji XD, Fearon ER: **Extinction of E-cadherin expression in breast cancer via a dominant repression pathway acting on proximal promoter elements**. *Oncogene* 1999, **18**:7274-7279.
47. Ji XD, Woodard AS, Rimm DL, Fearon ER: **Transcriptional defects underlie loss of E-cadherin expression in breast cancer**. *Cell Growth Diff* 1997, **8**:773-778.
48. Cano A, Perez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, Portillo F, Nieto MA: **The transcription factor Snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression**. *Nat Cell Biol* 2000, **2**:76-83.
49. Batlle E, Sancho E, Franci C, Dominguez D, Monfar M, Baulida J, de Herreros AG: **The transcription factor Snail is a repressor of E-cadherin gene expression in epithelial tumour cells**. *Nat Cell Biol* 2000, **2**:84-89.
50. Somasiri A, Howarth A, Goswami D, Dedhar S, Roskelley CD: **Overexpression of the integrin-linked kinase mesenchymally transforms mammary epithelial cells**. *J Cell Sci* 2001, **114**:1125-1136.
51. Tan C, Costello P, Sanghera J, Dominguez D, Baulida J, deHerreros AG, Dedhar S: **Inhibition of integrin linked kinase (ILK) suppresses beta-catenin-Lef/Tcf-dependent transcription and expression of the E-cadherin repressor, snail, in APC–/– human colon carcinoma cells**. *Oncogene* 2001, **20**:133-140.
52. D'Souza B, Taylor-Papadimitriou J: **Overexpression of erb-B2 in human mammary epithelial cells signals inhibition of transcription of the E-cadherin gene**. *Proc Natl Acad Sci USA* 1994, **91**:7202-7206.
53. Jhappan C, Geiser AG, Kordon EC, Bagheri D, Hennighausen L, Roberts AB, Smith GH, Merlino G: **Targeting expression of a transforming growth factor-beta-1 transgene to the pregnant mammary gland inhibits alveolar development and lactation**. *EMBO J* 1993, **12**:1835-1845.
54. Miettinen PJ, Ebner R, Lopez AR, Derynck R: **TGF-beta induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors**. *J Cell Biol* 1994, **127**:2021-2036.
55. Piek E, Moustakas A, Heldin CH, Ten Dijke P: **TGF-b type I receptor/ALK-5 and Smad proteins mediate epithelial to mesenchymal transdifferentiation in NMuMG breast epithelial cells**. *J Cell Sci* 1999, **112**:4557-4568.
56. Comijn J, Berx G, Vermassen P, Verschueren K, van Grunsven L, Bruyneel E, Mareel M, Huylebroeck D, van Roy F: **The two-handed E-box-binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion**. *Mol Cell* 2001 **7**:1267–1278.