Editorial

Tumor suppressor gene(E-cadherin)and its role in normal and Malignant cells (Part Two)

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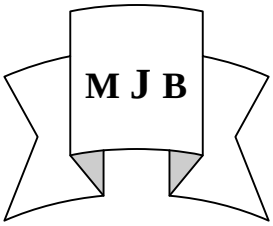

Role of Tumor suppressor gene(E-cadherin) in malignant cells

Progressive accumulation of somatic mutations in a number of different genes characterizes the process of tumorigenesis. Many genes involved in the process of tumorigenesis are components of one of a great many signal transduction pathways through which signals traffic via molecular networks. It is now apparent that epithelial malignancy can in certain aspects be explained by alterations in the adhesive properties of neoplastic cells.

Epithelial-mesenchymal conversion is also observed in malignant tumors of epithelial origin. This process is similar to developmental events but with the important difference that it is uncontrolled. Malignant carcinoma cells are characterized in general by poor intercellular adhesion, loss of the differentiated epithelial morphology and increased cellular motility. Downregulation or a complete shutdown of E-cadherin expression, mutation of the E-cadherin gene, or other mechanisms that interfere with the integrity of the adherens junctions, are observed in carcinoma cells. In human tumors, the loss of E-cadherin-mediated cell adhesion correlates with the loss of the epithelial morphology and with the acquisition of metastatic potential by the carcinoma cells [12]. Thus, a tumor invasion/suppressor role has been assigned to this gene. Additional data also support this role. Loss of heterozygosity on 16q is detected frequently in metastasizing malignancies derived from the liver, prostate, and breast [31]. Mutations in CDH1 have been described in a number of human cancers including breast, stomach, endometrium, ovary and thyroid [32,33]. Transgenic mouse model with loss of E-cadherin expression developed invasive carcinoma from well-differentiated adenomas [34] and finally germ-line mutations have recently been reported in early onset, diffuse-type stomach cancers [35].

Many immunohistochemical studies have examined changes in expression of the E-cadherin gene in human malignancies. In almost all non-

colonic tumors examined, the patterns of changes in the expression of this gene have been similar to that seen in colorectal cancer, i. e. loss of protein expression is positively correlated to loss of tumor differentiation. In oesophageal, pulmonary, squamous head and neck tumors, pancreatic and cervical tumors, loss of expression has been correlated with a high grade and an advanced stage of the disorder, with poor prognosis [2]. It has been reported that inactivating mutations of E-cadherin gene are highly frequent in infiltrating lobular breast carcinomas [38] and diffuse gastric carcinomas [32,33]. These mutations mostly occur in combination with loss of heterozygosity of the wild-type allele.

Another interesting paper [37] on E-cadherin expression demonstrates its reappearance in metastatic cells. Significant increase in re-expression of E-cadherin, together with alpha and beta-catenin, was observed in metastatic deposit of primary lobular breast cancers. This protein product was missing in the primary tumors of the same origin. Lobular breast cancers harbour E-cadherin mutations as well as losses of the gene locus, even *in situ* disease. That suggests that the mutated protein has a role even before invasion process started. The findings reveal another dimension of adhesion molecules in tumor metastasis: reestablishment of cellular contact may prevent apoptosis. I would like to cite M. Iiyas [38] "adhesion molecule expression is the phoenix in tumor metastasis".

And finally, let us not forget yet another level of CDH1 expression regulation, *i. e*. E-cadherin promotor hypermethylation. This process has been known to be an inactivating event in some tumors and is currently extensively researched [39].

Mutation reports

Most interesting genetic mutations and their consequences are listed in this section. Becker *et al*. [40] suggested that E-cadherin mutations contribute to the histopathologic appearance of stomach cancer because 13 of 26 diffuse gastric carcinomas, which had reduced homophilic cell-to-cell interactions, had abnormal gene transcripts that were not seen in non-cancerous tissue from same patients. Berx *et al*. [32] reported of 69 somatic mutations of the CDH1 gene. In addition to a few missense mutations, those were mainly splice site and truncation mutations caused by insertions, deletions, and nonsense mutations. There was a major difference in mutation type between diffuse gastric and infiltrative lobular breast cancers. In diffuse gastric tumors, the predominant defects were exon skippings, which caused in-frame deletions. In contrast, most mutations found in infiltrating lobular breast cancers were out-of-frame mutations, which yield secreted truncated E-cadherin products. In most cases these mutations occurred in combination with loss of heterozygosity.

Guilford *et al*. [35] reported germline mutations in the CDH1 gene in 3 familial gastric cancer kindreds of Maori origin from New Zealand. Furthermore, Richards *et al*. [41] analyzed 8 UK gastric cancer kindreds and identified novel germline CDH1 mutations (a nonsense and a splice site mutation] in 2 families. Both mutations were predicted to truncate the E-cadherin protein in the signal peptide domain. Gayther *et al*. [42] also described germline CDH1 mutations in familial gastric cancer. In a family with a strong history of diffuse gastric carcinoma, Chun *et al*. [43] found the 1558insC germline mutation in the CDH1 gene. The gastric cancer was of the early onset, histologically diffuse type. In another family with early onset diffuse gastric cancer, Guilford *et al*. [35] found that the 30-year-old proband was heterozygous for A-to-T transition at nucleotide 2095, which resulted in a

nonsense mutation. The mutation was predicted to result in an E-cadherin peptide lacking both the transmembrane and cytoplasmic domains. Same authors [35] described a family in which multiple members with gastric cancer were heterozygous for the insertion of an additional C residue in a run of 5 cytosines at positions 2382 to 2386. The resulting frameshift led to an E-cadherin molecule lacking about half of its cytoplasmic domain. In another diffuse gastric cancer family, a heterozygous G-to-T transversion at nucleotide 70 in exon 2 of the CDH1 gene, was also identified. The mutation converted a glutamic acid (glu24) to a TAG stop codon in the signal peptide of the E-cadherin precursor protein.

Richards *et al*. [41] identified a splice acceptor site mutation, an A-to-G transition at position -2 from nucleotide 49 at the start of exon 2 of the CDH1 gene and also a germline G-to-A transition at nucleotide 59 in exon 2. The mutation, a trp20-to-ter substitution was predicted to truncate the E-cadherin gene product in the signal peptide domain, which is cleaved from the N terminus of the mature protein.

In a family segregating diffuse gastric cancer, Gayther *et al*. [42] found a 2095C-T transition in the CDH1 gene, resulting in a truncating mutation, arg598 to ter.

Same authors [42] found a 1-bp insertion in the CDH1 gene in the proband of a family with familial diffuse gastric cancer. Insertion of a G after nucleotide 1711 created a frameshift that would truncate the protein at codon 587. Another 1-bp insertion (C) at nucleotide 1588 in exon 11 was also identified in a family segregating diffuse gastric cancer.

Mutations were also found in other types of carcinoma. In an endometrial carcinoma, Risinger *et al*. [44] identified a GCA-to-ACA transition in codon 617 predicting a substitution of thr for ala in the E-cadherin molecule. Somatic loss of heterozygosity was identified in the tumor tissue. They also identified a CTG-to-GTG transversion resulting in a leu711-to-val amino acid substitution in E-cadherin. The wild type allele was not lost.

In an ovarian carcinoma, same authors [44] identified an AGC-to-GGC transition in codon 838 resulting in substitution of glycine for serine. The tumor tissue also showed somatic loss of heterozygosity. In an infiltrative lobular breast carcinoma, Berx et al. [1] found a GAA (glu)-to-TAA (stop) nonsense mutation in the CDH1 gene. Tumor-specific loss of heterozygosity of chromosomal region 16q22.1 was demonstrated in this case. Here only selected examples of CDH1 mutations in carcinoma tissue have been assessed. The number of mutations is growing each day .

Pećina-Šlaus *et al*. [45] indicated yet another type of genomic instability of CDH1 gene encountered in tumor tissue. When searching for allelic loss i. e. Loss of Heterozygosity (LOH) of the CDH1 gene in clear cell renal cell carcinoma, they came across Replication ERror (RER) positive samples, an instability problem [46] that characterizes tumor development and progression. Replication/repair machinery seems to be targeted in 10% of clear cell renal cell carcinoma sample.

Conclusions

Reduced expression of E-cadherin is regarded as one of the main molecular events involved in dysfunction of the cell-cell adhesion system, triggering cancer invasion and metastasis. Therefore, E-cadherin is an important tumor suppressor gene. Research on E-cadherin has elucidated insights into both embryogenesis and oncogenesis. One of the most crucial exhibit of E-cadherin's function in development is the controlled epithelial - mesenchymal conversion.

The involvement of E-cadherin in wnt signaling, indicates that same molecule may have different functions and that E-cadherin can regulate cellular response generated by external signals the cell receives. In this way it can regulate migration, proliferation, apoptosis and cell differentiation.

The method of blocking E-cadherin downregulation in tumors is one of the important future approaches in gene therapy. To target this molecule is the logical path to prevent metastazing potential of almost any epithelial tumor. Nevertheless, it will not be an easy enterprise since its downregulation is caused by many different mechanisms, ranging from mutations and gross deletions to repression of gene transcription, as well as signal transduction stimulation of E-cadherin adhesion complex formation.

References

1. Berx G, Staes K, van Hengel J, Molemans F, Bussemakers MJ, van Bokhoven A, van Roy F: Genomics 1995, 26:281.
2. Guilford P: Mol Med Today, 1999, 5: 172.
3. Gumbiner BM: Cell adhesion: Cell 1996, 84:345.
4. Barth AI, Nathke IS, Nelson WJ: Curr Opin Cell Biol 1997, 9:683.
5. Gallin WJ, Sorkin BC, Edelman GM, Cunningham BA: Proc Natl Acad Sci USA 1987, 84:2808.
6. Ringwald M, Baribault H, Schmidt C, Kemler R: Nucleic Acids Res 1991, 19:6533.
7. Mansouri A, Spurr N, Goodfellow PN, Kemler R: Differentation 1998, 38:67.
8. Vleminckx K, Vakaet Jr L, Mareel M, Fiers W, van Roy F: Cell 1991, 66:107.
9. Bussemakers MJG, van Bokhoven A, Völler M, Smit FP, Schalken JA: Biochem Biophys Res Commun 1994, 203:291.
10. Kaupmann K, Becker-Follmann J, Scherer G, Jockusch H, Starzinski-Powitz A: Genomics 1992, 14:488.
11. Humphries MJ, Newham P: Trends Cell Biol 1998, 8:78.
12. Rietmacher D, Brinkmann V, Birchmeier CA: Proc Natl Acad Sci USA 1995, 92:855.
13. Yap AS, Niessen CM, Gumbiner BM: J Cell Biol 1998, 141:779.
14. Huber AH, Weis WI: Cell 2001, 105:391.
15. Takeichi M: Cadherins in cancer:Curr Opin Cell Biol 1993, 5:806.
16. Xiang YY, Tanaka M, Suzuki M, Igarashi H, Kiyokawa E, Naito Y, Othaware Y, Shen Q, Sugimura H, Kino I: Cancer Res 1994, 54:3034.
17. Berndorff D, Gessner R, Kreft B, Schnoy N, Lajouspetter AM., Loch N, Reutter W, Hortsch M, Tauber R: J Cell Biol 1994, 125:1353.
18. Schneider R: The human protooncogene ret: Trends Biochem Sci 1992, 17:468.
19. Iwamoto T, Taniguchi M, Asai N, Ohkusu K, Nakashima I, Takahashi M: Oncogene 1993, 8:1087.
20. Larue L, Ohsugi M, Hirchenhain J, Kemler R: Proc Natl Acad Sci USA 1994, 91:8263.
21. Fleming T, Hay M, Javed Q: Development Suppl 1992, 17:105.
22. Hyafil F, Morello D, Babinet C, Jacob F: Cell 1980, 21:927.
23. Vestweber D, Kemler R: Exp Cell Res 1984, 152:169.
24. Sefton M, Johnson M., Clayton L: Development [Cambridge, UK] 1992, 115:313.
25. Thiery JP: Nat Rev Cancer 2002, 2:442.
26. Babb SG, Barnett J, Doedens AL, Cobb N, Liu Q, Sorkin BC, Yelick PC, Raymond PA, Marrs JA: Dev Dyn 2001, 221:231.
27. Hulsken J, Birchmeier W, Behrens J: J Cell Biol 1994, 127:2061.

28. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW: Science 1997, 275:1787.
29. Pece S, Gutkind JS: J Biol Chem 2000, 275:41227.
30. Peifer M, Polakis P: Science 2000, 287:1606.
31. Efstathiou JA, Liu D, Wheeler JMD, Kim HC, Beck NE, Ilyas M., Karayiannakis AJ, Mortensen NJMcC, Kmiot W, Playford RJ, Pignatelli M, Bodmer WF: PNASOnline 1999, 96:2316.
32. Berx G, Becker KF, Hofler H, van Roy F: Hum Mutat 1998, 12:226.
33. Machado JC, Soares P, Carneiro F, Rocha A, Beck S, Blin N, Berx G, Sobrinho-Simoes M: Lab Invest 1999, 79:459.
34. Christofori G, Semb H: Trends Biochem Sci 1999, 24:73.
35. Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harrawira P, Taite H, Scoular R, Millers A, Reeve A: Nature 1998, 392:402.
36. Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harrawira P, Taite H, Scoular R, Millers A, Reeve A: Nature 1998, 392:402.
37. Huiping C, Sigurgeirsdottir JR, Jonasson JG, Eiriksdottir G, Johannsdottir JT, Egilsson V, Ingvarsson S: Br J Cancer 1999, 81:1103.
38. Bukholm IK, Nesland JM, Børessen-Dale AL: J Pathol 2000, 190:15-19.
39. Ilyas M: J Pathol 2000, 190:3.
40. Tycko B:J Clin Invest 2000, 105:401.
41. Becker KF, Atkinson MJ, Reich U, Becker I, Nekarda H, Siewert JR, Hoefler H: Cancer Res 1994, 54:3845.
42. Richards FM, McKee SA, Rajpar MH, Cole TRP, Evans DGR, Jankowski JA, McKeown C, Sanders DSA, Maher ER:Hum Mol Genet 1999, 8:607.
42. Gayther SA, Gorringe KL, Ramus SJ, Huntsman D, Roviello F, Grehan N, Machado JC, Pinto E, Seruca R, Halling K, MacLeod P: Hum Mol Genet 1999, 8:607.
43. Chun YS, Lindor NM, Smyrk TC, Petersen BT, Burgart LJ, Guilford PJ, Donohue JH: Germline E-cadherin gene mutations: Cancer 2001, 92:181.
44. Risinger JI, Berchuck A, Kohler MF, Boyd J: Nature Genet 1994, 7:98.
45. Pećina-Šlaus N, Pavelić K, Pavelić J: J Mol Med 1999, 77:446.
46. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M Nives Pećina-Šlaus: Nature 1993, 363:558.

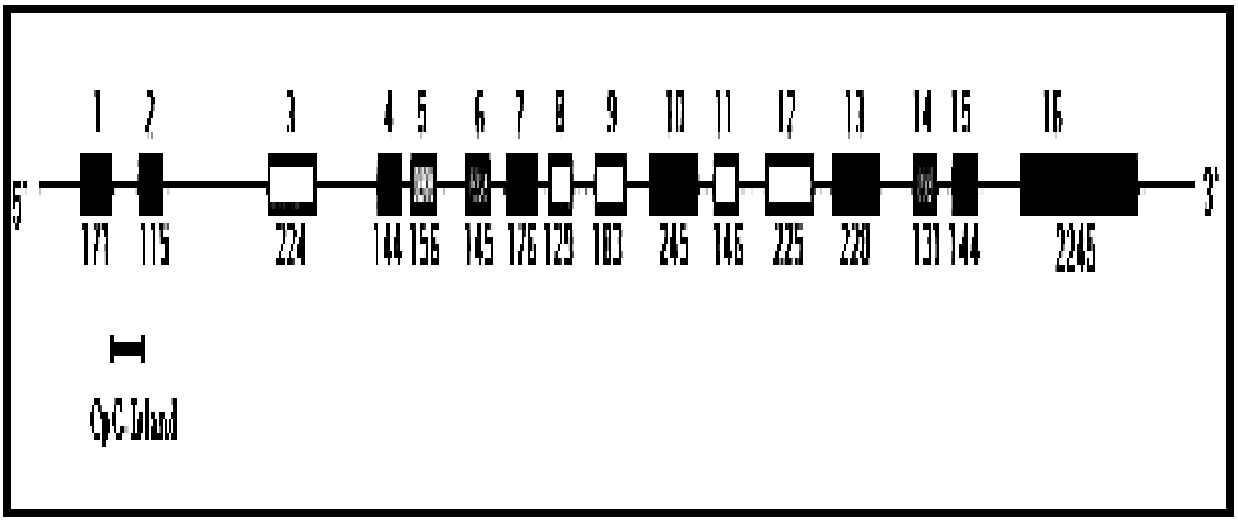

Fig1. Genomic organization of the human E-cadherin gene.

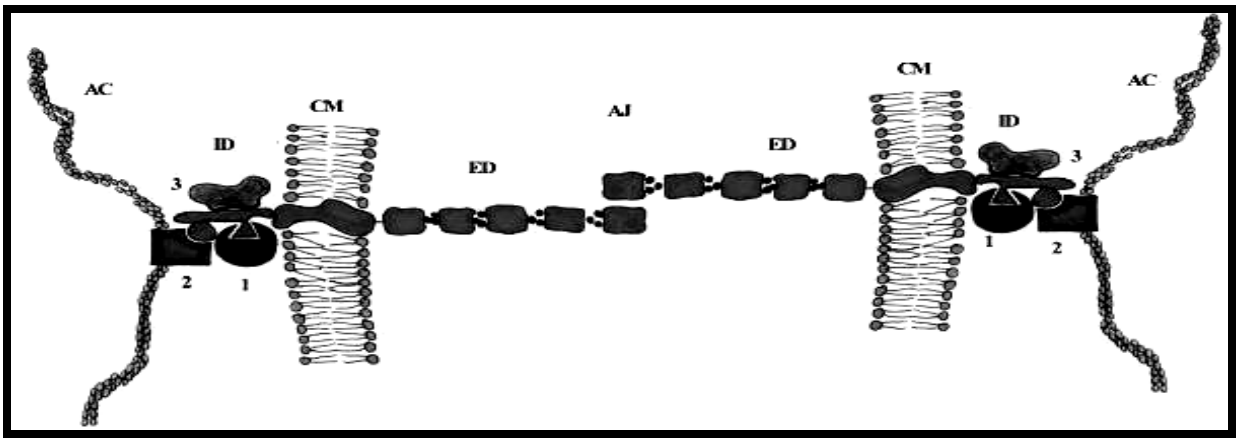

Fig 2 Shematic illustration of E-cadherin in adherens junction