Pergamon

European Journal of Cancer Vol. 31A, Nos. 7/8, pp. 1055–1060, 1995

0959-8049/95 $9.50+0.00

0959-8049(95)00128-X

DCC: Linking Tumour Suppressor Genes and Altered Cell Surface Interactions in Cancer?*

K.R. Cho and E.R. Fearon

The Deleted in Colorectal Cancer (*DCC*) gene is a candidate tumour suppressor gene encoding a neural cell adhesion molecule-like transmembrane protein. Over the last year, data supporting *DCC* inactivation in multiple tumour types have continued to accumulate. Functional studies suggest that *DCC* may participate in signalling pathways that regulate cell proliferation and/or differentiation, two cellular processes that often go awry during tumorigenesis.

Key words: tumour suppressor gene, deleted in colorectal cancer (*DCC*) gene, NCAMs, cell adhesion molecules in cancer, chromosome 18q, colorectal cancer biological features
Eur J Cancer, Vol. 31A, Nos 7/8, pp. 1055–1060, 1995

INTRODUCTION

DEFECTS in the expression and regulation of cell surface molecules may underlie many of the altered phenotypic properties of cancer cells, including their altered morphology, invasiveness, and ability to metastasise. Some genes encoding cell surface proteins that regulate cell–cell and/or cell–substrate interactions may be targeted for inactivation by inherited and/or somatic mutations in human cancer and as such, may be tumour suppressor genes. The expression of other tumour suppressors may be altered as a result of epigenetic mechanisms or defects in upstream regulatory genes. This review will focus on a chromosome 18q gene, termed *DCC* (for deleted in colorectal cancer), that may have an important role in effecting the altered cellular phenotype of cancer cells. We will review: (i) the identification and characterisation of *DCC*; (ii) the data suggesting that *DCC* inactivation may be a common event not only in colorectal cancers, but in a number of other tumour types; (iii) evidence suggesting that *DCC* inactivation may contribute to the ability of tumour cells to invade and metastasise; and (iv) the functional studies of *DCC* which support the notion that inactivation of *DCC* may lead to a loss of growth and/or differentiation control through a failure of the cells to respond to normal environmental cues provided by interactions at the cell surface.

Identification and characterisation of DCC

Tumour suppressor genes may be inactivated by several different mechanisms, including localised mutations or deletions of large chromosomal regions. Typically such deletions involve one of the two parental chromosome sets, or alleles, present in normal cells and are thus referred to as losses of heterozygosity (LOH) or allelic losses. In accord with an hypothesis originally proposed by Knudson [1], LOH usually unmasks a more subtle recessive mutation in the retained copy of a tumour suppressor gene.

A systematic study evaluating the prevalence of LOH of the non-acrocentric chromosomal arms in a series of colorectal tumours identified frequent LOH of chromosomes 5q, 17p and 18q [2, 3]. Specifically, allelic losses affecting 18q were detected in more than 70% of primary colorectal carcinomas, in approximately 50% of advanced adenomas, and infrequently in earlier stage adenomas. Several colorectal tumours that had LOH of some, but not all of the 18q markers were subsequently studied with additional 18q markers in order to localise a region of LOH common to each of these tumours, presumably containing the suppressor gene target [4]. Two tumours were found to have somatic mutations involving or immediately flanking an anonymous marker (*p15–65*) from within the commonly deleted region. These mutations included one tumour with loss of both copies of the 18q region containing *p15–65* (homozygous loss) and a second tumour with a point mutation that appeared to generate a novel splice acceptor site. Exons of *DCC* were subsequently identified in the region surrounding the *p15–65* marker. Sequencing of *DCC* cDNAs revealed that the gene encodes a 1447 amino acid transmembrane protein with significant similarity to the neural cell adhesion molecule (NCAM) family of cell surface proteins [4, 5].

The human *DCC* gene is very large, spanning greater than 1.35 million base pairs at chromosome band 18q21.1 [6], and including at least 29 exons [7]. *DCC* transcripts of approximately 12 kb have been identified by Northern blot analysis of adult and fetal brain tissues [4]. The *DCC* gene encodes a transmembrane protein with a single membrane spanning region separating the extracellular and cytoplasmic portions. The extracellular domain sequences bear strong similarity to those found in proteins

Correspondence to: K.R. Cho.
K.R. Cho is at the Department of Pathology, The Johns Hopkins University School of Medicine, Room 659 Ross Research Building, 720 Rutland Ave., Baltimore, MD 21205, U.S.A.; and E.R. Fearon is at the Department of Pathology and Program in Oncology and Development, Boyer Center for Molecular Medicine, Yale University School of Medicine, 295 Congress Ave., New Haven, CT 06536-0812, U.S.A.
* The material included in this article was originally published in *Current Opinion in Genetics & Development* 1995, 5, 72–78, and has been adapted with permission. © Current Biology Ltd.

involved in ligand binding or cell–cell interactions and include four immunoglobulin-like domains and six fibronectin type III-like motifs (Figure 1). The cytoplasmic domain of approximately 325 amino acids shares very little similarity to any other known proteins. *DCC* homologues have been identified and characterised in full or in part from other vertebrate species, including rat [4], chicken [8], and *Xenopus laevis* [9]. The predicted protein products are very well conserved (80–85% amino acid identity in *Xenopus*, 94% identity in chicken).

Alternatively spliced forms of *DCC* gene transcripts have been characterised [10]. In this regard, *DCC* is similar to other NCAM-like genes. For example, alternative splicing of NCAM transcripts has been identified in regions between the fibronectin type III-like domains, and is thought to introduce a "hinge" into the extracellular domain. Tissue-specific alternative splicing of a 20 codon region between the fourth and fifth fibronectin-like domains of *DCC* has been identified and may have similar functional consequences. In addition, a 6 bp region in the cytoplasmic domain of *DCC* has also been shown to be affected by alternative splicing. This alternative splice affects a consensus casein kinase II phosphorylation site and is similar to an alternatively spliced region in the cytoplasmic domain of L1. The functional consequences of the alternative splicing of *DCC* transcripts have yet to be determined.

Several studies have shown that the *DCC* gene is expressed, albeit at very low levels, in most normal adult tissues [4, 10]. Highest expression is observed in the central nervous system [5, 10]. Detection of *DCC* transcripts usually requires sensitive RT-PCR based analyses; however, in a subset of adult tissues, DCC protein has been detected by immunoblot and/or immunohistochemical stains. These tissues include rodent brain and bladder [10], *Xenopus* stomach, lung and kidney (Pierceall WE and Fearon ER, unpublished), human neural tissues and colonic epithelium [5], and several murine and avian tissues, including epithelia of the gut, skin, lung, mammary gland and bladder [8]. In many mature epithelia, *DCC* expression appears to be restricted to the proliferative compartment [8], suggesting that *DCC* may play a role in regulating cell proliferation.

DCC *appears to be frequently inactivated in colorectal cancers and other tumour types*

As discussed above, 18q losses of heterozygosity are present in the majority (>70%) of colorectal carcinomas. In greater than 90% of carcinomas with 18q allelic loss, the altered region includes the *DCC* locus. Moreover, marked reduction or loss of *DCC* mRNA expression is observed in more than 50% of primary colorectal cancers [11] and in 85% of colorectal tumour cell lines [4] suggesting that the retained *DCC* allele may be inactivated by a more localised mutation in many, if not the majority, of cases. Given the large size (>1350 kb) and complexity (29 exons with alternative splicing) of the gene, only a very small subset of *DCC* sequences have been systematically evaluated for localised mutations [7]. Nevertheless, somatic mutations have been identified in some cases. Approximately 10–15% of tumours have insertions in a microsatellite sequence located downstream of one of the *DCC* exons [4]. This finding

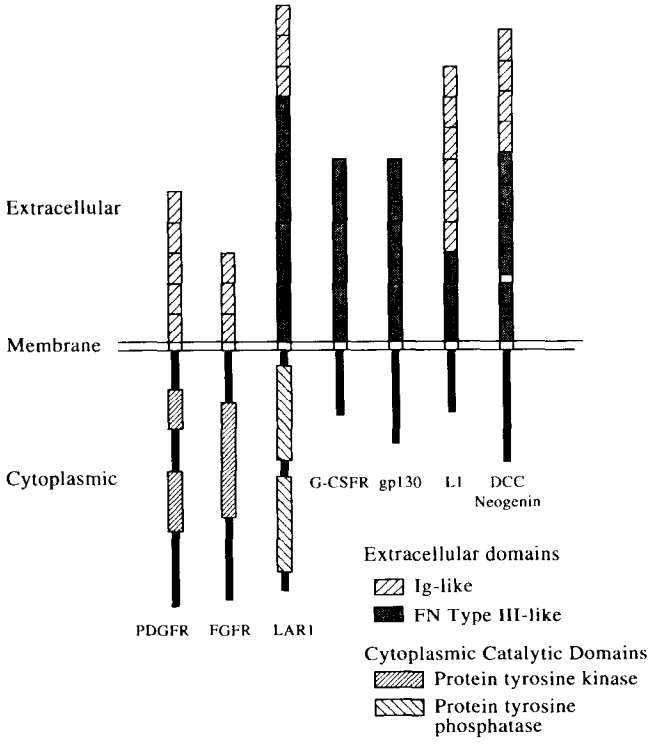

Figure 1. Schematic representation of the predicted structure of the DCC protein and selected transmembrane proteins containing immunoglobulin (Ig)-like domains. Growth factor receptors with Ig-like domains are shown, including the platelet-derived growth factor receptor (PDGFR) and fibroblast growth factor receptor (FGFR). A transmembrane tyrosine phosphatase known as the leucocyte antigen-related (LAR1) protein contains multiple Ig-like and FN type III-like extracellular domains and a protein tyrosine phosphatase cytoplasmic domain. Several other growth factor receptors, including the granulocyte colony stimulating receptor (G-CSFR) and the gp130 cytokine receptor dimeric partner protein, contain multiple FN type III-like domains and lack obvious catalytic domains in their cytoplasmic sequences. The structure of the NCAM-related molecule L1 is also shown. Neogenin is an avian cell surface protein closely related to DCC [47]. Like DCC, neogenin appears to play an important role in intercellular signalling and the regulation of neuronal differentiation.

is particularly intriguing in light of the more generalised instability of microsatellite sequences identified in a subset of sporadic colorectal cancers and in colorectal tumours of patients with the hereditary non-polyposis colorectal cancer (HNPCC) syndrome [12–14]. These insertions might affect *DCC* transcription or processing of transcripts, but functional support for this proposal has not yet been obtained. In addition, somatically acquired point mutations have been identified in three primary colorectal cancers [4, 7], including two mutations in introns and one in exon 28 (Pro → His).

Preliminary immunohistochemical studies have provided data suggesting that, although DCC protein expression is detectable in hyperplastic polyps and most adenomatous polyps of the colon, its expression is markedly reduced or absent in most colorectal cancers [5]. Mucinous carcinomas appear to retain *DCC* expression, perhaps in part, because they do not frequently exhibit 18q LOH [5]. Therefore, although the mechanisms (besides 18q LOH) underlying the loss of *DCC* expression have not yet been elucidated in most colorectal cancers, based on the loss of gene expression in the majority of cases and the somatic mutations identified in the gene, *DCC* remains the most promising candidate colorectal tumour suppressor gene identified to date on chromosome 18q.

Recently, mounting evidence has been obtained that *DCC* may be inactivated in several tumours arising outside the colon and rectum, including epithelial tumours of the stomach, pancreas, endometrium, breast, prostate, oesophagus, and bladder, as well as in male germ cell tumours, some leukaemias, and gliomas. The data providing evidence for *DCC* inactivation in non-colorectal tumours are summarised in Table 1. Support for *DCC* inactivation in these tumours includes frequent LOH of the portion of chromosome 18q harbouring the *DCC* gene, markedly reduced or absent *DCC* mRNA expression, aberrantly sized *DCC* transcripts, homozygous loss of *DCC* in several cases, and in a few cases, intragenic point mutations. Notably, one of the two homozygous deletions affecting the *DCC* gene in a panel of 91 male germ cell tumours resulted in complete loss of the 3′ region of the *DCC* gene, but did not affect most 5′ exons [15]. In contrast, a homozygous deletion of *DCC* identified in a primary colorectal cancer resulted in complete loss of the 5′ region of *DCC* (exons 1–12), with one copy of chromosome 18q harbouring *DCC* exons 13–29 retained in the tumour [4]. If each of the homozygous losses provided a growth advantage to the tumour cells because they inactivated the same tumour suppressor gene(s) on 18q, then the only possible candidate genes include *DCC* and any still to be identified genes that may be contained within *DCC* introns.

DCC *inactivation may contribute to the ability of cancer cells to invade and/or metastasise*

Patients whose colorectal cancers have 18q LOH have an increased likelihood of distant metastasis and death from their disease [16]. Recent studies have provided evidence that 18q allelic loss (presumably including loss of *DCC*) may be a strong predictive factor for deep muscle and lymphatic invasion and hepatic metastasis [17] and for death in patients with stage II cancers [18]. These findings suggest that colorectal tumours with 18q LOH tend to behave more aggressively and metastasise more readily. This notion is underscored by the fact that the prevalence of 18q LOH rises to nearly 100% in colorectal

Table 1. *Evidence for* DCC *inactivation in tumours arising outside the colon and rectum*

Cancer type	Summary of data	References
Gastric carcinoma	18q LOH in 61%	Uchino *et al.* [31]
Pancreatic carcinoma	Decreased or absent *DCC* expression in 50%	Hohne *et al.* [32]
	18q LOH in 80%	Seymour *et al.* [33]
Breast carcinoma	Decreased, absent, or altered transcripts in 70%	Thompson *et al.* [34]
	18q LOH in 30%	Devilee *et al.* [35]
Prostate carcinoma	18q LOH in 26–45%	Latil *et al.* [36], Gao *et al.* [37], Brewster *et al.* [38]
	Decreased or absent *DCC* expression in 86%	Gao *et al.* [36]
Endometrial carcinoma	18q LOH in 26–40%	Imamura *et al.* [39], Gima *et al.* [40]
	Reduced or absent *DCC* expression in 50%	Gima *et al.* [40]
Oesophageal carcinoma	18q LOH in 23–24%	Miyake *et al.* [41], Huang *et al.* [42]
	Somatically acquired missense point mutation in metastatic lesion	Miyake *et al.* [41]
Bladder carcinoma	18q LOH in 35%, association with muscle invasive disease	Brewster *et al.* [20]
Gliomas	Reduced or absent *DCC* expression in 68% (86% in glioblastomas)	Scheck and Coons [43]
Male germ cell tumours	18q LOH in 45%	
	Homozygous loss of *DCC* (2/91 cases)	Murty *et al.* [15]
	Reduced or absent *DCC* expression in 29%	
Leukaemias	Reduced or absent *DCC* expression in 24–33%	Miyake *et al.* [44]
	Homozygous loss of *DCC* (1/64 cases)	Miyake *et al.* [44]
	Loss of *DCC* expression in all 4 cases with monosomy 18	Porfiri *et al.* [45]
	Reduced or absent *DCC* expression in 71% of overt leukaemias arising from myelodysplastic syndrome	Miyake *et al.* [46]

carcinoma metastases to the liver [17, 19]. In another study, all four colorectal carcinomas associated with liver metastases showed decreased *DCC* mRNA expression [11]. Moreover, data suggesting that *DCC* inactivation contributes to aggressive tumour behaviour are not limited to studies of colorectal tumours. In bladder carcinomas, allelic loss at the *DCC* locus is associated with muscle invasive disease in a statistically significant manner [20].

Functional studies of the DCC *gene*

Many different classes of cell-surface proteins, including integrins, cadherins, and immunoglobulin superfamily cell adhesion molecules (CAMs), are known to have critical roles in embryonic development, the differentiation of various cell types, and the regulation of cell proliferation [21–23]. DCC bears the most similarity to the neural cell adhesion branch of the immunoglobulin superfamily of cell-surface proteins [4, 5]. While several of the immunoglobulin superfamily CAMs mediate cell adhesion through homotypic interactions, others function through heterotypic interactions between opposing cell surfaces. Furthermore, immunoglobulin-like domains and/or fibronectin type III-like repeats characterise several other cell-surface proteins that function as growth factor receptors, rather than as cell adhesion molecules. Although preliminary studies from one group suggest that DCC may mediate Ca^{2+}-independent cell aggregation *in vitro* through heterotypic interactions [8], others have not had success in demonstrating DCC-mediated cell aggregation in various *DCC* transfected cell lines (Ekstrand BC and Fearon ER, unpublished). Thus, although DCC may function as a cell adhesion molecule, further studies will be necessary to firmly establish this proposal. Moreover, the extremely low levels of *DCC* expression in most adult tissues suggest that DCC is unlikely to function generally in cell–cell or cell–matrix adhesiveness. Rather, it seems more likely that DCC may function in specific signalling processes that regulate cell proliferation and/or differentiation, although ligands and/or receptors for DCC have not yet been identified.

Recent studies of the expression of *DCC* in chick, mouse, and frog embryonic development have provided some support for the proposal that *DCC* may regulate proliferation and differentiation in dividing cell populations. Collectively, these studies also provide some insights into the mechanisms by which *DCC* gene inactivation may contribute to tumorigenesis. During chick and mouse early embryonic development, *DCC* is expressed in all epithelial cell layers of the gut, skin, mammary glands, lung, and urinary bladder [8]. However, as the epithelia mature, *DCC* expression apparently becomes limited to proliferating cells (e.g. intestinal crypts and basal layers of squamous epithelia), and is markedly reduced or absent in differentiated cell types. In some tissues, DCC may function, at least in part, via epithelial–mesenchymal interactions. Studies utilising an embryonic chicken skin explant culture model of feather bud development suggest that DCC function in epithelia is essential for the condensation of underlying mesenchymal cells leading to the formation of feather placodes [8].

DCC expression in *Xenopus* embryos has been shown to vary dynamically during development [9]. *DCC* is induced during neurulation and rises throughout the developmental stages in which brain segmentation and neural cell specification take place. The expression is then markedly reduced prior to metamorphosis. In the developing neural tube, *DCC* expression does not appear to be continuous, but is restricted to certain regions of the hind-, mid-, and forebrain. This restricted pattern of gene expression appears to correspond to the compartmentalisation that is known to occur in the developing vertebrate neural tube. Furthermore, *DCC* expression is seen predominantly in the intermediate and dorsal regions of the developing neural tube, and not in the floorplate or notochord. The regions in which *DCC* is most highly expressed have been proposed to contain both proliferating and differentiating cells [24]; thus it appears that *DCC* may play an important role in regulating cell specification and/or proliferation in the developing nervous system. As *DCC* is also expressed in some mature vertebrate tissues, it appears that *DCC* may regulate proliferation and differentiation of selected cell populations in the adult as well. Inactivation of *DCC* function in these cells could thus contribute to several of the phenotypic properties often recognised in tumour cells, namely, loss of growth control and aberrant differentiation.

Other studies support the notion that *DCC* may have a critical functional role in differentiation pathways and cell fate determination. PC12 is a rat adrenal pheochromocytoma cell line that can be induced to differentiate to a sympathetic neuronal phenotype in response to nerve growth factor (NGF), fibroblast growth factor, or a subset of extracellular matrix components and cell adhesion molecules. NGF-mediated morphological differentiation of PC12 cells is inhibited in cells transfected with and expressing high levels of an antisense *DCC* expression construct or when the PC12 cells are incubated with antisense *DCC* oligonucleotides [25]. Other studies have shown that NIH3T3 cells expressing the full-length DCC protein can stimulate morphological differentiation of PC12 cells through signalling pathways distinct from those utilised in NGF-mediated differentiation [26]. Thus, while the data from both sets of studies suggest a role for DCC in mediating differentiation of PC12 cells, the findings suggest that DCC may have a complex role in the process. The antisense studies suggest that DCC may function in some sense as a receptor on the PC12 cells, while the studies utilising DCC-expressing NIH3T3 cells suggest that DCC may function as a ligand for an as yet undefined receptor on PC12 cells.

Some of the strongest evidence supporting *DCC*'s role as a tumour suppressor gene has been obtained from studies showing suppression of tumorigenicity by reconstitution of DCC function in tumour cells lacking *DCC* expression. Introduction of chromosome 18 (and presumptively wild-type *DCC*) into COKFu colon carcinoma cells resulted in suppression of growth in soft agar and suppression of tumorigenicity in nude mice [27]. More recent studies directly implicate *DCC* as the gene on chromosome 18q responsible for this effect, in at least some cases. Expression of full length, but not truncated DCC, in transformed keratinocytes lacking DCC expression suppressed tumorigenicity of these cells in nude mice [28, 29]. Furthermore, tumorigenic reversion of initially suppressed transfectants was associated with loss of DCC expression and loss or rearrangement of transfected *DCC* sequences.

A number of tumour suppressor genes have been identified and characterised thus far, and it is interesting to note that their protein products appear to function in several different cellular compartments, including the nucleus (e.g. p53, p105RB, and WT1), the cell cytoplasm (e.g. APC and NF-1), and at the cell surface (e.g. NF-2, DCC). A large body of evidence has established that tumours arise from the accumulation of multiple genetic alterations [30]. In a somewhat similar manner, it seems reasonable to expect that in any given tumour type, alterations of signalling pathways in several different cellular compartments may be required to bring about the fully malignant phenotype.

DCC is a cell surface protein that is differentially expressed during development, and is expressed in specific subsets of cells in mature tissues. In some tissues, such as gut and cutaneous epithelia, DCC expression appears to be primarily restricted to the proliferative compartment. In others, such as the central nervous system, DCC expression is noted primarily in differentiated cells. Inactivation of DCC may thus have different consequences in different cell types, and it is hoped that further studies will identify the means by which DCC inactivation may contribute to the altered properties of cancer cells, with respect to aberrant growth control and loss of differentiation.

CONCLUSION

Many reports have detailed the phenotypic alterations observed in cancer cells, including changes in cell morphology and tissue architecture, loss of differentiated phenotype, decreased cell adhesion and aggregation, increased motility, and invasive behaviour. The molecular events underlying these alterations in cell phenotype have yet to be fully understood, however, cell surface proteins that mediate cellular responses to environmental cues through cell–cell or cell–matrix interactions are likely to be among the targets for inactivation during tumorigenesis. A candidate tumour suppressor gene termed *DCC* has been identified within the region of chromosome 18q commonly deleted in colorectal cancers. The gene encodes a transmembrane protein with strong structural similarity to NCAM. Expression of this gene is markedly decreased or absent in the majority of colorectal cancers and cell lines, and somatic mutations within the *DCC* gene have been observed in a subset of cases. Thus, *DCC* represents the strongest candidate tumour suppressor gene on 18q identified to date. Additional studies should enhance our understanding of *DCC* function in developing and mature tissues and more conclusively establish its role in tumour suppression.

1. Knudson A. Hereditary cancer, oncogenes and antioncogenes. *Cancer Res* 1985, **45**, 1437–1443.
2. Vogelstein B, Fearon E, Kern S, *et al.* Allelotype of colorectal carcinomas. *Science* 1989, **244**, 207–211.
3. Vogelstein B, Fearon E, Hamilton S, *et al.* Genetic alterations during colorectal tumor development. *N Engl J Med* 1988, **319**, 525–532.
4. Fearon ER, Cho KR, Nigro JM, *et al.* Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science* 1990, **247**, 49–56.
5. Hedrick L, Cho KR, Fearon ER, Wu TC, Kinzler KW, Vogelstein B. The DCC gene product in cellular differentiation and colorectal tumorigenesis. *Genes Devel* 1994, **8**, 1174–1183.
6. LeBeau M, Overhauser J, Straub R, *et al.* Report of the first international workshop on human chromosome 18 mapping. *Cytogenet Cell Genet* 1993, **63**, 78–96.
7. Cho KR, Oliner JD, Simons JW, *et al.* The DCC gene — structural analysis and mutations in colorectal carcinomas. *Genomics* 1994, **19**, 525–531.
8. Chuong CM, Jiang TX, Yin E, Widelitz RB. cDCC (chicken homologue to a gene deleted in colorectal carcinoma) is an epithelial adhesion molecule expressed in the basal cells and involved in epithelial–mesenchymal interaction. *Devel Biol* 1994, **164**, 383–397.
9. Pierceall WE, Reale MA, Candia AF, Wright CVE, Cho KR, Fearon ER. Expression of a homologue of the deleted in colorectal cancer (*DCC*) gene in the nervous system of developing *Xenopus* embryos. *Devel Biol* 1994, **166**, 654–665.
10. Reale MA, Hu G, Zafar AI, Getzenberg RH, Levin SM, Fearon ER. Expression and alternative splicing of the deleted in colorectal cancer (*DCC*) gene in normal and malignant tissues. *Cancer Res* 1994, **54**, 4493–4501.
11. Itoh F, Hinoda Y, Ohe M, *et al.* Decreased expression of DCC mRNA in human colorectal cancers. *Int J Cancer* 1993, **53**, 260–263.
12. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993, **260**, 816–819.
13. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 1993, **363**, 558–561.
14. Aaltonen L, Peltomaki P, Leach F, *et al.* Clues to the pathogenesis of familial colorectal cancer. *Science* 1993, **260**, 812–816.
15. Murty VVVS, Li RG, Houldsworth J, *et al.* Frequent allelic deletions and loss of expression characterize the DCC gene in male germ cell tumors. *Oncogene* 1994, **9**, 3227–3231.
16. Kern S, Fearon E, Tersmette K, *et al.* Allelic loss in colorectal carcinomas. *JAMA* 1989, **261**, 3099–3103.
17. Iino H, Fukayama M, Maeda Y, *et al.* Molecular genetics for clinical management of colorectal carcinoma. *Cancer* 1994, **73**, 1324–1331.
18. Jen J, Kim H, Piantadosi S, *et al.* Chromosome 18q loss and prognosis of colorectal cancer. *N Engl J Med* 1994, **331**, 213–221.
19. Ookawa K, Sakamoto M, Hirohashi S, *et al.* Concordant p53 and DCC alterations and allelic losses on chromosomes 13q and 14q associated with liver metastases of colorectal carcinoma. *Int J Cancer* 1993, **53**, 382–387.
20. Brewster SF, Gingell JC, Browne S, Brown KW. Loss of heterozygosity on chromosome 18q is associated with muscle-invasive transitional cell carcinoma of the bladder. *Br J Cancer* 1994, **70**, 697–700.
21. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* 1992, **69**, 11–25.
22. Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* 1991, **251**, 1451–1455.
23. Edelman G, Crossin K. Cell adhesion molecules: implications for a molecular histology. *A Rev Biochem* 1991, **60**, 155–190.
24. Hartenstein V. Early neurogenesis in *Xenopus*: the spatio-temporal pattern of proliferation and cell lineages in the embryonic spinal cord. *Neuron* 1989, **3**, 399–411.
25. Lawlor K, Narayanan R. Persistent expression of the tumor suppressor gene DCC is essential for neuronal differentiation. *Cell Growth Different* 1992, **3**, 609–616.
26. Pierceall WE, Cho KR, Getzenberg RH, *et al.* NIH3T3 cells expressing the deleted in colorectal cancer tumor suppressor gene product stimulate neurite outgrowth in rat pc12 pheochromocytoma cells. *J Cell Biol* 1994, **124**, 1017–1027.
27. Tanaka K, Oshimura M, Kikuchi R, Seki M, Hayashi T, Miyaki M. Suppression of tumorigenicity in human colon carcinoma cells by introduction of normal chromosome 5 or 18. *Nature* 1991, **349**, 340–342.
28. Klingelhutz AJ, Smith PP, Garrett LR, McDougall JK. Alteration of the DCC tumor-suppressor gene in tumorigenic HPV-18 immortalized human keratinocytes transformed by nitrosomethylurea. *Oncogene* 1993, **8**, 95–99.
29. Klingelhutz AJ, Hedrick L, Cho KR, McDougall JK. The DCC gene suppresses the malignant phenotype of transformed human epithelial cells. *Oncogene* 1995, **10**, 1581–1586.
30. Fearon E, Vogelstein BA. Genetic model for colorectal tumorigenesis. *Cell* 1990, **61**, 759–767.
31. Uchino S, Tsuda H, Noguchi M, *et al.* Frequent loss of heterozygosity at the *DCC* locus in gastric cancer. *Cancer Res* 1992, **52**, 3099–3102.
32. Hohne MW, Halatsch ME, Kahl GF, Weinel RJ. Frequent loss of expression of the potential tumor suppressor gene *DCC* in ductal pancreatic adenocarcinoma. *Cancer Res* 1992, **52**, 2616–2619.
33. Seymour AB, Hruban RH, Redston M, *et al.* Allelotype of pancreatic adenocarcinoma. *Cancer Res* 1994, **54**, 2761–2764.
34. Thompson AM, Morris RG, Wallace M, Wyllie AH, Steel CM, Carter DC. Allele loss from 5q21 (APC/MCC) and 18q21 (DCC) and DCC mRNA expression in breast cancer. *Br J Cancer* 1993, **68**, 64–68.
35. Devilee P, van Vliet M, Kuipers-Dijkshoorn N, Pearson P, Cornelisse C. Somatic genetic changes on chromosome 18 in breast carcinomas: is the DCC gene involved? *Oncogene* 1991, **6**, 311–315.
36. Gao X, Honn K, Grignon D, Sakr W, Chen Y. Frequent loss of expression and loss of heterozygosity of the putative tumor suppressor gene DCC in prostatic carcinomas. *Cancer Res* 1993, **53**, 2723–2727.
37. Brewster SF, Browne S, Brown KW. Somatic allelic loss at the DCC, APC, NM23-h1 and p53 tumor suppressor gene loci in human prostatic carcinoma. *J Urol* 1994, **151**, 1073–1077.
38. Latil A, Baron J-C, Cussenot O, *et al.* Genetic alterations in localized

prostate cancer: identification of a common region of deletion on chromosome arm 18q. *Genes Chrom Cancer* 1994, **11**, 119–125.
39. Imamura T, Arima T, Kato H, Miyamoto S, Sasazuki T, Wake N. Chromosomal deletions and K-ras gene mutations in human endometrial carcinomas. *Int J Cancer* 1992, **51**, 47–52.
40. Gima T, Kato H, Honda T, Imamura T, Sasazuki T, Wake N. DCC gene alteration in human endometrial carcinomas. *Int J Cancer* 1994, **57**, 480–485.
41. Miyake S, Nagai K, Yoshino K, Oto M, Endo M, Yuasa Y. Point mutations and allelic deletion of tumor suppressor gene DCC in human esophageal squamous cell carcinomas and their relation to metastasis. *Cancer Res* 1994, **54**, 3007–3010.
42. Huang Y, Boynton RF, Blount PL, *et al.* Loss of heterozygosity involves multiple tumor suppressor genes in human esophageal cancers. *Cancer Res* 1992, **52**, 6525–6530.
43. Scheck AC, Coons SW. Expression of the tumor suppressor gene DCC in human gliomas. *Cancer Res* 1993, **53**, 5605–5609.
44. Miyake K, Inokuchi K, Dan K, Nomura T. Alterations in the deleted in colorectal carcinoma gene in human primary leukemias. *Blood* 1994, **82**, 927–930.
45. Porfiri E, Secker-Walker L, Hoffbrand A, Hancock J. DCC tumor suppressor gene is inactivated in hematologic malignancies showing monosomy 18. *Blood* 1993, **81**, 2696–2701.
46. Miyake K, Inokuchi K, Dan K, Nomura T. Expression of the DCC gene in myelodysplastic syndromes and overt leukemia. *Leukemia Res* 1993, **17**, 785–788.
47. Vielmetter J, Kayyem JF, Roman JM, Dreyer WJ. Neogenin, an avian cell surface protein expressed during terminal neuronal differentiation, is closely related to the human tumor suppressor molecule delected in colorectal cancer. *J Cell Biol* 1995, **127**, 2009–2020.