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Invited Review

Expression of differentiation-related genes in colorectal cancer: possible implications for prognosis

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Summary. Although differentiation grade is an important prognostic factor for colorectal tumors, its usefulness is limited since its predictive value for tumor behavior is not very significant. This might be related to the subjective nature of histological assessment of differentiation grade, which allows the distinction of only three grades, and with limited reproducibility. Characterization of the differentiation process at the biochemical level may improve our understanding of normal and malignant differentiation, and is expected to provide molecular markers with higher discriminative potential than histomorphology. Several studies have compared gene expression in undifferentiated and differentiated colon carcinoma cells, and many differentially expressed genes have been identified. Some of these, including HLA class I, nucleophosmin, adenylosuccinate lyase, α-tubulin, and a novel gene designated Drg1, were found to be expressed at different levels in neoplastic as compared to normal tissue. In this review the rationale, implementation, and results of this approach are discussed, as well as the characteristics of two novel differentially expressed genes, ICT1 (previously named DS-1) and Drg1.

Key words: Colon epithelium, Colorectal carcinoma, Differentiation, Marker, Myeloid, ICT1, Drg1

Introduction

Billions of colon epithelial cells are shed into the colon lumen daily. The high turnover relies on rapid proliferation and differentiation of the colon epithelium, which appears to be organized hierarchically. Stem cells located at the crypt bottom divide infrequently and asymmetrically, presumably to minimize the accumulation of replication errors in the stem cell compartment. The daughter cell that is destined to differentiate subsequently enters a rapid proliferation phase and migrates upward in the direction of the intestinal lumen. When the upper half of the crypt is reached, proliferation ceases and differentiation ensues (for reviews see Podolsky, 1993; Gordon and Hermiston, 1994; Potten et al., 1997). The hierarchical organization of proliferation and differentiation at the cellular level may underlie the observation that colon carcinomas, which by histological criteria are classified as poorly differentiated (and therefore contain cells with high growth potential), generally show a worse prognosis than well-differentiated tumors (Kronborg, 1993). However, the histological assessment of differentiation is limited to only three grades (poorly, moderately, and well differentiated) and subject to significant inter-observer variability. Rather than by a subjective morphological impression, differentiation might also be assessed through the analysis of the expression of differentiation-related molecular markers that may be visualized by immunohistochemistry. Elucidation of the biochemical pathways underlying morphological differentiation could provide a range of new molecular markers that may improve our understanding of normal and malignant cell differentiation, and, ultimately, patient stratification for additional therapy. This review discusses the prognostic value of colon epithelial cell differentiation markers for colorectal cancer. In addition, the characteristics of two differentiation-related genes recently cloned in our lab are summarized and updated. The genetic abnormalities underlying colorectal carcinogenesis, mucin-related antigens, and tumor-associated antigens, have recently been excellently reviewed elsewhere (Grogan et al., 1996).

Differentiation markers

A rational approach for the analysis of differentiation in colorectal cancer has been the study of markers of differentiation related with the cell lineages that can be distinguished in colorectal mucosa. For stem cells, few reliable markers have been described. Proliferation is not limited to stem cells, and proliferation markers

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therefore do not label the stem cell population only (Kikuchi et al., 1997). Likewise, bcl-2 immunoreactivity, however limited it might be to crypt base cells, is not limited to stem cells only (Flohil et al., 1996). As has been outlined above, stem cell markers would be of interest in the perspective of differentiation in colorectal cancer because in theory clonogenic (stem cell like) rather than terminally differentiated cells are responsible for tumor progression.

For endocrine cells a whole range of markers is available, including the chromogranin family, synaptophysin, serotonin, and specific neurohormonal peptides. Investigation of the expression patterns of specific neuroendocrine markers has shown that about one out of five colorectal tumors shows neuroendocrine differentiation (Jansson et al., 1988). Some authors have reported that carcinomas with neuroendocrine features are more aggressive than carcinomas without (Hamada et al., 1992; De Bruine et al., 1993), but this has been contested by others (Ferrero et al., 1995).

For goblet cells a range of markers is also available. Early studies were done using conventional mucin stains, such as high-iron diamine alcian blue (HID-AB) (Lev et al., 1985). By this approach it was found that tumors in a mucosa with production of sialomucins recur more frequently than those with predominant production of sulphomucins (Habib et al., 1986). Later studies have focused more on immunohistochemically identifiable mucin markers. In general, useful correlations between specific mucin markers (such as MUC-1 and MUC-2) (Ogata et al., 1992) have not been found. A recent paper (van der Wurff et al., 1998) reports on a MUC-2 epitope, recognized by a monoclonal antibody (5E9), that is normally expressed in crypt bases but also in some colorectal tumors rather extensively. Expression of this epitope correlated in Dukes B tumors with more aggressive tumor behavior.

For columnar cells or enterocytes a variety of markers is also available. Specific enzymes, such as sucrase-isomaltase (Wiltz et al., 1991), dipeptidyl-peptidase IV (formerly also known as adenosine deaminase complexing protein, Ten Kate et al., 1985), or aminopeptidase N (Quaroni et al., 1992), are associated with the brush border, as is the cytoskeletal protein villin (Ho, 1992). Secretory component, the specific membrane receptor for dimeric IgA and responsible for transcellular transport of secretory IgA, is expressed only in columnar cells (Huang et al., 1976). Of most of these markers the prognostic significance has been studied. A limited impact has been found for some markers (e.g. secretory component (Arends et al., 1984)), but in general terms, the presence of columnar cell differentiation does not appear to have an important bearing on tumor behavior. An interesting study was published by Ho et al., who did not analyze single markers but combined the markers for various lineages. The general conclusion of this study was that with more markers of differentiation, tumor behavior tended to be more favorable (Ho et al., 1989).

In general terms, in spite of the clinicohistopathological and -immunohistopathological correlations found, clinical utility of all these markers remains limited. For many markers, multivariate analyses to test for independent predictive value have not been done, and for those that have thus been analysed the conclusion was usually that prognostic value failed to reach a level of significance that would make the marker useful in daily practice. As a consequence, given the fact that tumor differentiation clearly bears a biologically relevant message which might have a clinical impact, the search has been on for new markers of differentiation.

Molecular strategies for the development of new differentiation markers

The available molecular technology allows for the detection of differential gene expression e.g. by differential screening of cDNA libraries (Hoog, 1991), subtraction hybridization (review Sagerstrom et al., 1997), differential display of RNA (Liang and Pardee, 1992; Welsh et al., 1992; Van Belzen et al., 1997), serial analysis of gene expression (SAGE) (Velculescu et al., 1995), and DNA microarray hybridization (Schena et al., 1995). The best approach appears to be the comparison of gene expression in two cell populations that differ only in the level of differentiation. Alternatively, gene expression could be compared in highly differentiated and poorly differentiated carcinomas, but different tumors may differ in many respects, not necessarily restricted to differentiation. For this reason, cell lines that can be induced to differentiate or normal crypt epithelium fractionated according to stage of differentiation appear more attractive starting points. Since isolation of pure populations of normal colon epithelial cells at different stages of differentiation is not feasible presently, we have used in vitro differentiation of the human colon carcinoma cell line HT29-D4 (Fantini et al., 1986; Rabenandrasana et al., 1990) as a model (Zweibaum et al., 1991). Using differential screening, subtraction hybridization, and differential display of mRNA, we have identified several genes that are differentially expressed during maturation.

Genes found to be induced during differentiation include ATPase6, HLA-B (Van Belzen et al., 1995), and Drg1 (Van Belzen et al., 1997). Interestingly, the expression of these genes is decreased in colorectal neoplasms as compared to normal colon mucosa. It remains to be determined whether the decrease simply reflects the decreased differentiation of malignant cells, or whether it confers a selective advantage to the tumor. Decreased expression of HLA-B and other HLA class I genes might lower the susceptibility of tumor cells to immune attack by cytotoxic T lymphocytes; on the other hand, it increases the likelihood of lysis by natural killer cells. Notwithstanding the potential immunomodulatory effects of HLA class I, its expression does not appear to be a prognostic factor in colorectal carcinoma (Moller et al., 1991). The reduced expression of mitochondrial

genes like ATPase6 and cytochrome oxidase III in colorectal cancer (Augenlicht and Heerdt, 1992) may be related to decreased apoptosis in the transformed cells (Bedi et al., 1995; Heerdt et al., 1996). We are not aware of studies that determine the prognostic significance of mitochondrial gene expression in colorectal cancer.

Genes downregulated during in vitro differentiation include Hsc70, adenylosuccinate lyase, nucleophosmin, α-tubulin, and ICT1 (Van Belzen et al., 1995). In malignant as compared to normal cells, an increase was found in adenylosuccinate lyase activity (Jackson et al., 1977; Reed et al., 1987), α-tubulin levels (Taguchi et al., 1991), and nucleophosmin levels (Nozawa et al., 1996); the increased expression of these genes in tumors may relate to the higher proliferation rate of neoplastic cells. These data underscore the usefulness of the in vitro colon carcinoma cell line differentiation model to identify genes that are differentially expressed in malignant as compared to normal cells.

The strongest downregulation during in vitro differentiation of HT29-D4 colon carcinoma cells was observed for a novel gene designated ICT1 (Fig. 1, Van Belzen et al., 1995), whereas the strongest induction was observed for a novel gene designated Drg1 (differentiation-related gene 1, Fig. 1) (Van Belzen et al., 1997). The modulation of ICT1 and Drg1 appears to be related to the differentiation process and not to the concomitant decrease in proliferation, since their expression is hardly influenced during confluency-induced proliferation inhibition of A431 cells (Van Belzen et al., 1995, 1997). The ICT1 and Drg1 genes were further characterized by cloning and sequencing of the complete cDNA, their chromosomal localization was determined, and antisera were raised against ICT1 and Drg1 proteins.

The ICT1 gene

According to the guidelines for human gene nomenclature (White et al., 1997), the gene previously designated DS-1 (EMBL/Genbank accession number X81788) has been renamed to the HUGO/GDB approved symbol ICT1 (immature colon carcinoma cell transcript 1).

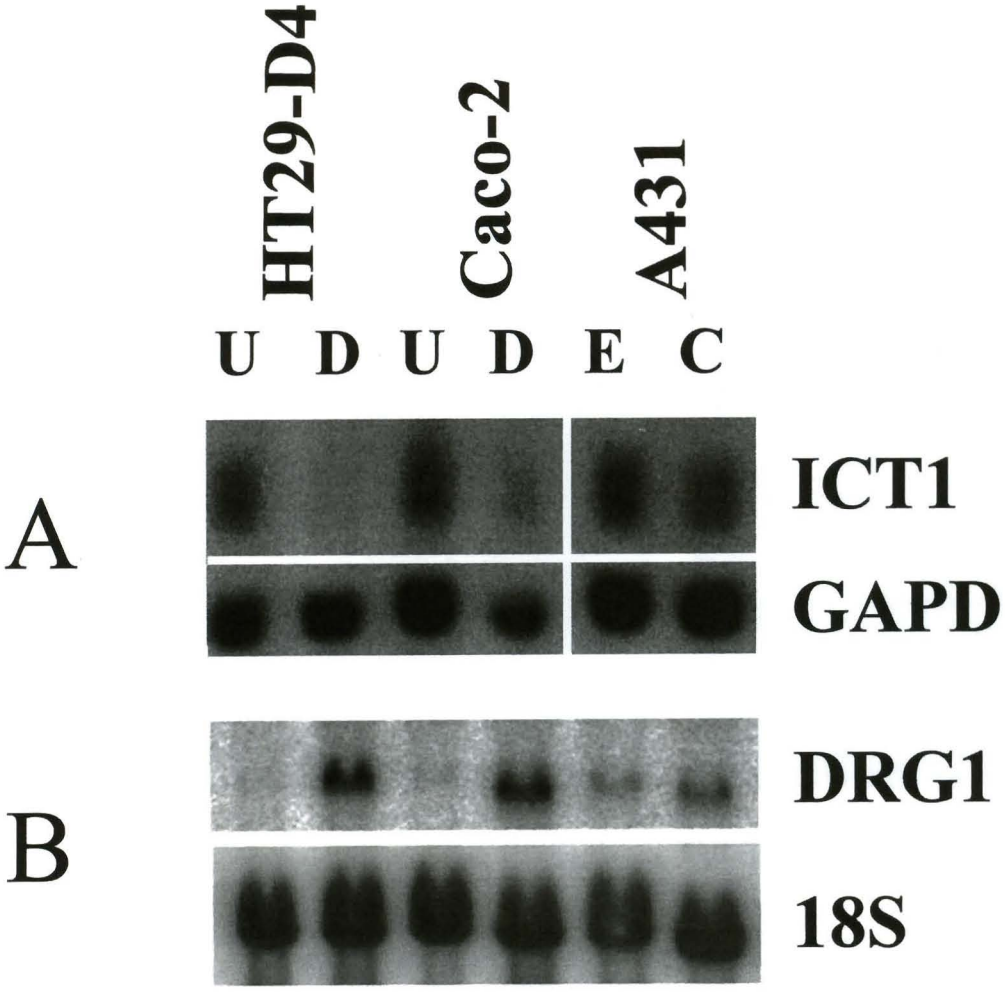

Fig. 1. Modulation of ICT1 and Drg1 mRNA expression during differentiation of colon carcinoma cells in vitro. RNA isolated from undifferentiated (U) and differentiated (D) HT29-D4 and Caco-2 colon carcinoma cells was Northern blotted, and hybridized to radiolabeled ICT1 **(A)** and Drg1 **(B)** cDNA as described (Van Belzen et al., 1995, 1997). Subsequently, blots were stripped and reprobed with glyceraldehyde phosphate dehydrogenase (GAPD) or 18S cDNA to quantify the amount of RNA present in the different lanes. RNA isolated from exponentially (E) or confluently (C) grown A431 human epidermoid carcinoma cells served as a control for proliferation-related expression differences. Fig. 1A and 1B are reproduced from Van Belzen et al. (1995) and Van Belzen et al. (1997), respectively, with permission.

The 0.9 kb ICT1 mRNA is downregulated 5-fold during in vitro differentiation of HT29-D4 colon carcinoma cells. The ICT1 transcript encodes a basic 24 kDa protein that is also downregulated during in vitro differentiation. ICT1 mRNA was hardly detectable on Northern blots prepared from normal and neoplastic colon mucosa, indicating that in vivo expression levels are low. Low to undetectable expression of ICT1 mRNA in other adult human tissues is suggested by Northern blotting experiments (Van Belzen et al. unpublished), as well as by the modest representation of ICT1 in human expressed sequence tag (EST) databases. Unfortunately, histological localization of the ICT1 protein was not possible, because the anti-ICT1 serum (prepared by immunizing rabbits with ICT1 peptide) was not reactive on frozen or paraffin tissue sections (Van Belzen et al., 1995). The human ICT1 gene was localized to chromosome 17q25 by fluorescent in situ hybridization (Fig. 2A).

Since alignment of a human protein with its homologues in other organisms may provide clues as to its functional domains, that may be conserved during evolution, DNA libraries from model organisms were searched to identify proteins homologous to human ICT1.

Assembly and conceptual translation of mouse EST sequences that display high homology to ICT1 cDNA yielded a putative mouse amino acid sequence of the same length as human ICT1 protein, that displays 79% identity and 88% similarity to human ICT1 (Fig. 3). In

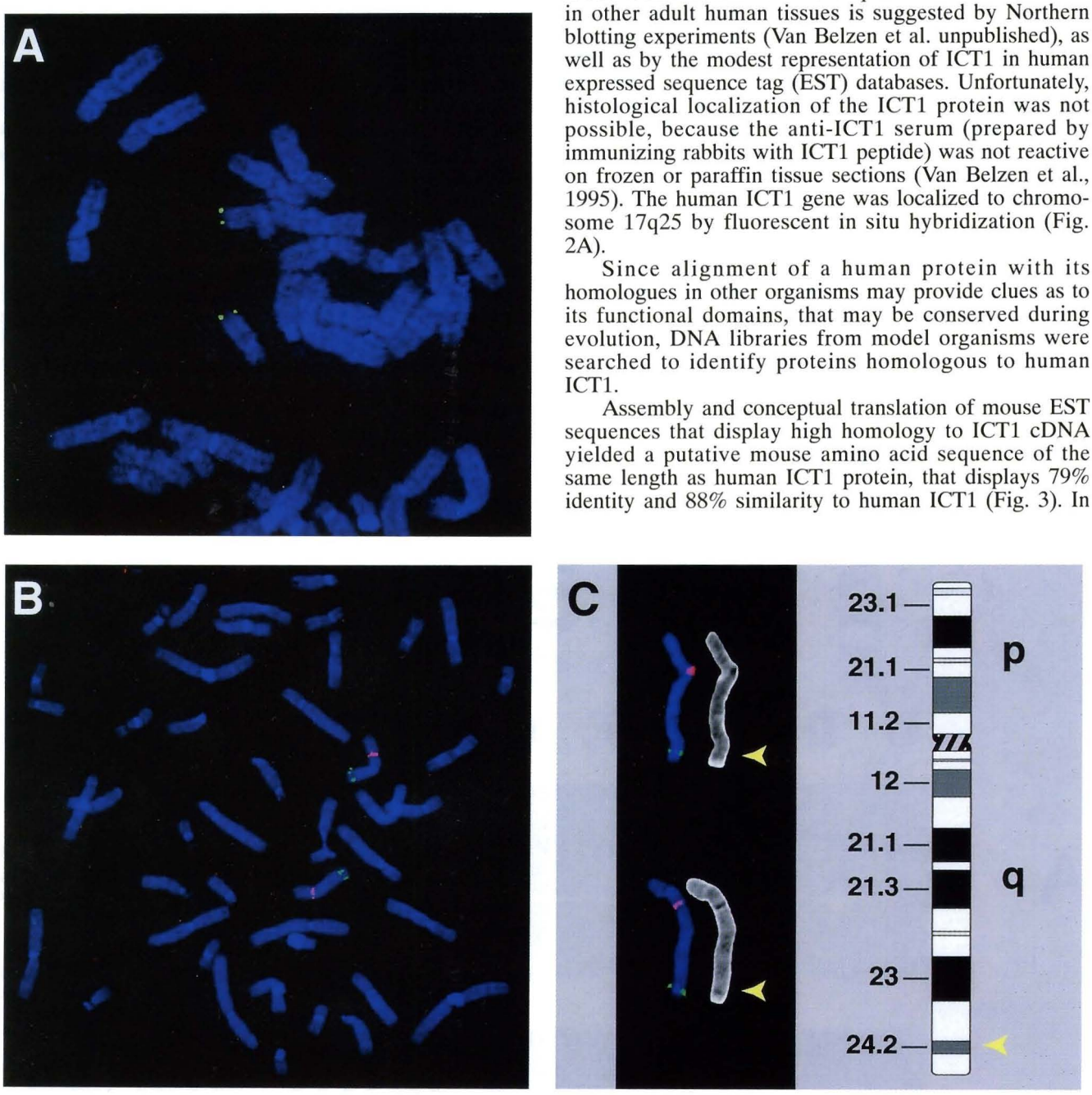

Fig. 2. Chromosomal localization of the ICT1 and Drg1 genes. **A.** A genomic ICT1 clone was isolated by screening a lambda human genomic library with the ICT1 cDNA. The genomic clone was partially sequenced to confirm the presence of the ICT1 gene, and mapped using standard FISH techniques (Lichter et al., 1990; Wiegant et al., 1991). Briefly, the probe was biotinylated by nick translation, hybridized to metaphase spreads of cultured normal human peripheral blood lymphocytes, stained by two alternating layers of avidin-FITC, and localized to 17q25 by fluorescence microscopy. **B.** To map the Drg1 gene, a human P1 artificial chromosome (PAC) library was screened with the Drg1 cDNA. Two overlapping PACs were obtained that yielded identical mapping results. The PAC shown here was digoxygenin-labeled by nick translation, hybridized as described above, and detected by a FITC-labeled anti-digoxygenin antibody. The probe for chromosome 8 centromer (D8Z2, (Donlon and al., 1986)) was labeled by direct incorporation of the fluorochrome tetramethylrhodamine. **C.** The PAC was localized to 8q24.2 in a combined inverted DAPI gray-scale image.

the mouse EST database, ICT1 is more frequently represented than in the corresponding human database. Strikingly, approximately half of the ESTs that match the ICT1 sequence were cloned from a cDNA library prepared from 13.5-14.5-day-old mouse embryos, suggesting that ICT1 is highly expressed during a discrete stage of embryonic development and only moderately in adult tissues. In the Caenorhabditis elegans database the strongest match to ICT1 was observed for a predicted protein designated R02F2.2 (EMBL/Genbank accession number U00055, Wilson et al., 1994) that consists of 492 amino acids, of which the 208 C-terminal residues display 29% identity and 41% similarity to human ICT1 (Fig. 3). In *Saccharomyces cerevisiae* (budding yeast), the highest homology to ICT1 was observed for the predicted protein designated YOL114C (EMBL/Genbank Z74856, MIPS European yeast chromosome XV sequencing project), that contains 202 amino acids and shows 28% identity and 40% similarity with human ICT1 (Fig. 3). Expression of YOL114C mRNA does not change during the shift of yeast from glucose fermentation to ethanol-fueled respiration (DeRisi et al., 1997), which may indicate that the decrease in glucose concentration in the culture medium used in HT29-D4 cell differentiation does not directly influence ICT1 expression. In bacteria, no ICT1 homologues were observed, although domains similar to the ICT1 motif SGPGGQNV were found in several proteins, including peptidase D, peptide chain release factor, and beta-ketoadipate:succinyl-CoA transferase. Since this amino acid sequence is also completely conserved in the human, mouse, *C. elegans*, and yeast ICT1 proteins (Fig. 3), it may comprise a domain essential for the function of ICT1.

Being the transcript most strongly downregulated during in vitro differentiation of colon carcinoma cell lines in our studies, ICT1 appears a promising candidate marker for undifferentiated colon epithelial cells. The low expression of ICT1 mRNA and protein in adult tissues and the poor reactivity of the anti-peptide serum on tissue sections have limited experimental work on ICT1. The clue derived from the mouse genome project that ICT1 may be highly expressed during mouse embryogenesis strengthens the suggestion that ICT1 expression may be related to an immature cellular phenotype and warrants further study, such as in situ hybridization on mouse embryos, and efforts to raise an antiserum (e.g. against ICT1 fusion protein) that can immunolocalize human ICT1 protein in histological sections. As a potential marker for undifferentiated cells, ICT1 might provide a valuable tumor marker, since its expression is expected to positively correlate with tumor aggressiveness.

The Drg1 gene

The Drg1 transcript is induced 20-fold during differentiation of HT29-D4 and Caco2 colon carcinoma cell lines, and downregulated 6-fold in colorectal carcinomas as compared to normal colon mucosa. The mRNA encodes an acidic 43 kDa protein that is virtually absent in colon epithelial crypt cells, but strongly expressed in the epithelial cells that border the colon lumen. Well differentiated colon carcinomas showed focal expression of Drg1 protein, whereas poorly differentiated tumors showed weak diffuse expression

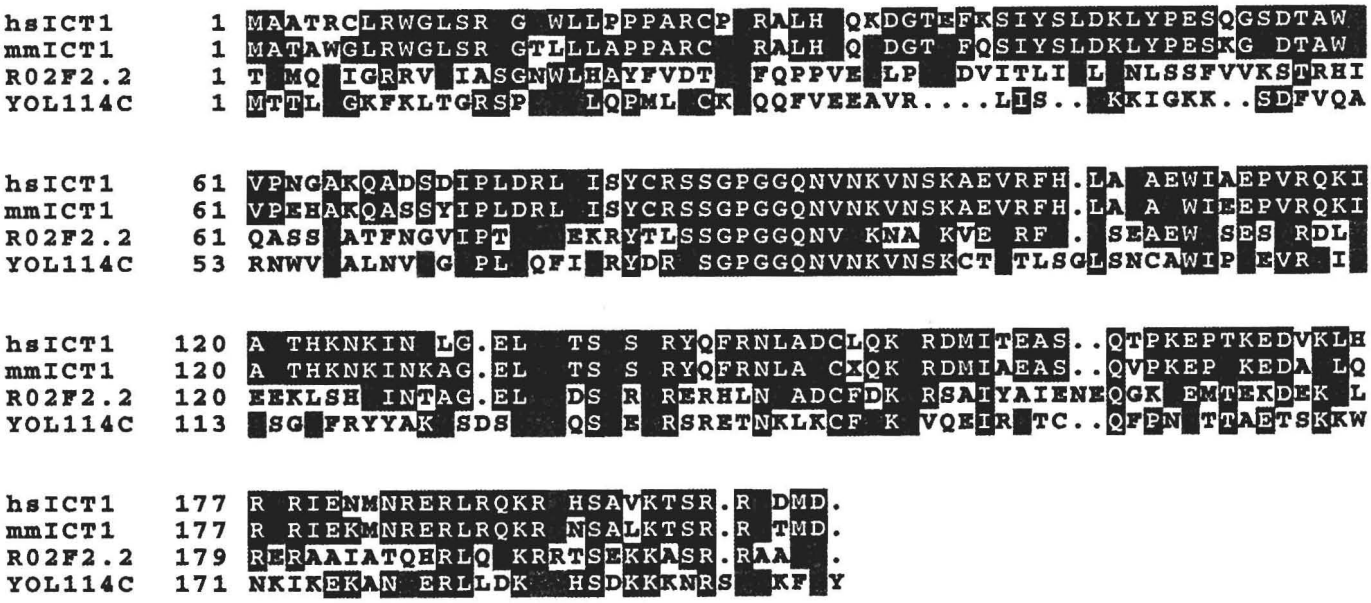

Fig 3. Alignment of the ICT1 protein with homologous proteins from mouse, *C. elegans*, and yeast. The putative mouse homologue was constructed by conceptual translation of assembled expressed sequence tags. Of the *C. elegans* protein, only the C-terminal domain is shown. Alignment was performed using the program ClustalW (Thompson et al., 1994) and printed using Boxshade.

(Van Belzen et al., 1997). The Drg1 gene was mapped to human chromosome 8q24.2 by fluorescent in situ hybridization (Fig. 2B,C), in agreement with PCR-based mapping data from the Radiation Hybrid Mapping Consortium (Généthon Cda0zg03; Sanger stSG4268).

Modulation of Drg1 expression during myeloid differentiation was suggested by EMBL/Genbank database searching with the Drg1 cDNA, that revealed a 120 bp EST (EMBL/Genbank D20948, Okubo et al. unpublished) with high homology to the 3' end of the

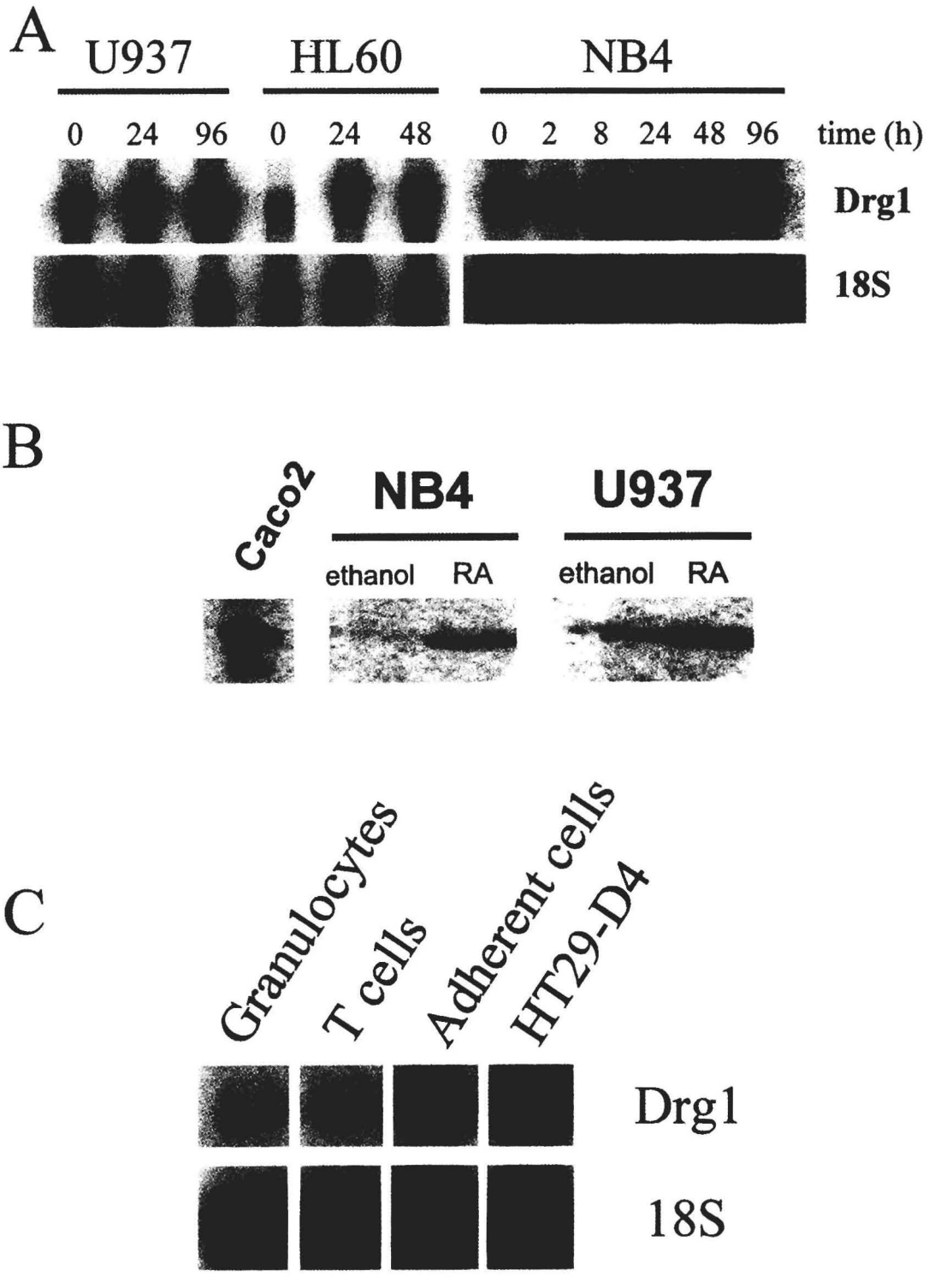

Fig. 4. Expression of Drg1 mRNA and protein in hematopoietic cells.
A. The myeloid cell lines HL60, NB4, and U937 were treated with 1 μM all-trans retinoic acid (RA) for the time indicated. RNA was Northern blotted and hybridized to radiolabeled Drg1 cDNA as described (Van Belzen et al., 1997). Subsequently, blots were stripped and reprobed with radiolabeled 18S cDNA to quantify the amount of RNA present in the different lanes.
B. NB4 and U937 cells were treated with 1 mM RA for 48 h. As a control, cells were treated with solvent (ethanol) alone. Western blotting with anti-Drg1 antiserum was performed as described (Van Belzen et al., 1997).
C. Granulocytes, T cells, and adherent cells were isolated from a buffy coat from a healthy blood donor using Ficoll centrifugation, T cell rosetting, and adherence to tissue culture plastic. RNA was isolated, and Drg1 mRNA was detected by Northern hybridization.

Drg1 cDNA. According to the sequence annotation, the EST had been isolated from the human promyelocytic cell line HL60 before or after induction of differentiation. This prompted us to investigate the expression of Drg1 during retinoic acid-induced differentiation of the myeloid cell lines HL60, NB4 and U937. As shown in Fig. 4A, Drg1 mRNA expression was induced by retinoic acid in all three cell lines. Standardized to 18S RNA expression, induction in HL60 appeared lower (4-fold) than in the other two cell lines (7-fold). Basal Drg1 transcript levels appeared higher in U937 cells. Drg1 protein expression was studied in NB4 and U937 and found to be induced as well (Fig. 4B). To determine Drg1 mRNA levels in normal peripheral blood cells, a buffy coat from a healthy donor was fractionated by Ficoll centrifugation, T cell E-rosetting, and adherence to tissue culture plastic. Drg1 mRNA levels in granulocytes and adherent cells (monocytes) were found to be 1.3- and 9-fold higher, respectively, than in T cells. The Drg1 mRNA level in monocytes was relatively high, approximately half of that in HT29-D4 cells (Fig. 4C). The higher Drg1 expression in monocytes than in granulocytes may relate to the cell line data that indicate that Drg1 mRNA induction in NB4 is transient (Fig. 4A). Drg1 mRNA levels in normal bone marrow were quite low. The Drg1 transcript was also detected in bone marrow from acute myeloid leukemia, acute lymphoblastic leukemia, and chronic lymphocytic leukemia patients (not shown).

The Drg1 gene has independently been cloned from several other model systems. Strong and rapid Drg1 mRNA induction was observed in homocysteine-treated human vascular endothelial cells by Kokame et al. (1996), who used the designation RTP. A mouse gene designated TDD5, that shows high homology to Drg1, was found to be repressed by androgens in a T cell hybridoma (Lin and Chang, 1997). Mouse Drg1 has also been identified as a gene named Ndr1 reported to be downstream of N-myc (EMBL/Genbank U60593, A. Shimono and H. Kondoh, unpublished).

The Drg1 gene appears to be expressed in all tissues examined thus far, and has been suggested to serve a housekeeping function (Kokame et al., 1996). However, in other studies it was noted that some tissues, including human placental membranes and prostate (Van Belzen et al., 1997), and mouse kidney (Lin and Chang, 1997), showed much higher levels of Drg1 mRNA than others. Moreover, Drg1 expression is increased upon

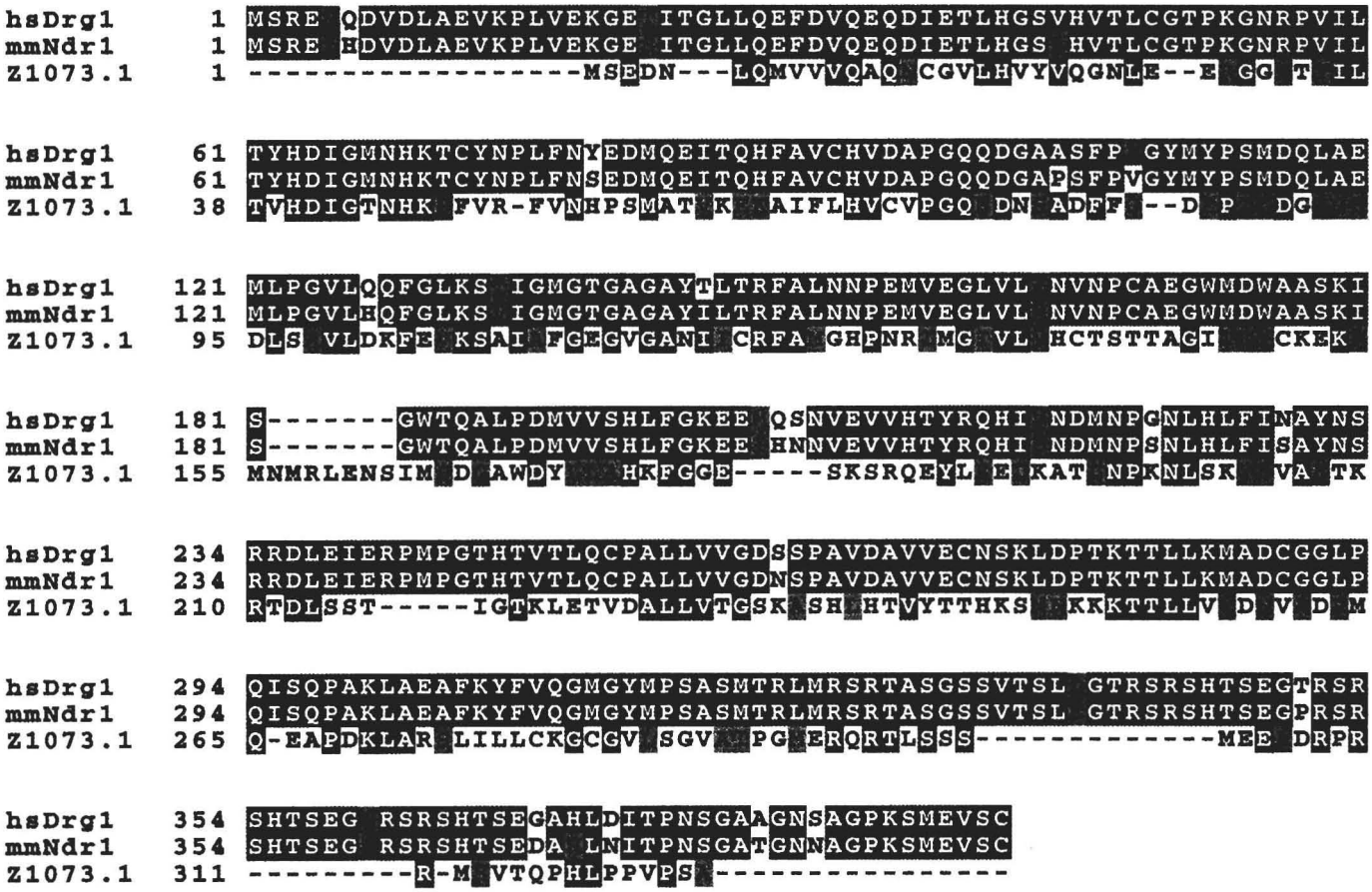

Fig. 5. Alignment of Drg1 with homologous proteins from mouse and *C. elegans*. Alignment was performed as described in the legend of Fig. 2.

differentiation of colon epithelial (Van Belzen et al., 1997) and myeloid cells (Fig. 4), and modulated by several agents, including androgens (Lin and Chang, 1997), reducing agents, tunicamycin (Kokame et al., 1996), and retinoic acid (Fig. 4). The expression data therefore argue against a housekeeping role for Drg1; a basic biochemical function is also unlikely since we have not been able to identify a Drg1 homologue in yeast.

Alignment of Drg1 with its mouse homologue Ndr1 reveals high conservation of the amino acid sequence, with 94% identity and 96% similarity. The *C. elegans* homologue (Z1073.1, EMBL/Genbank Z68135, Nematode Sequencing Project) shows 27% identity and 43% similarity to Drg1 protein (Fig. 5). No particular regions with higher than average homology were seen in the *C. elegans* homologue as compared to the amino acid sequences of human and mouse Drg1.

The function of the Drg1 protein is still unclear. Its localization to the plasma membrane of luminal colon epithelial cells just prior to cell shedding suggests that it may be involved in the regulation of cell-cell contact (Van Belzen et al., 1997). The high Drg1 expression observed in monocytes may relate to the ability of these cells to form and disrupt contacts with substratum and other cells. On the other hand, Drg1 may be involved in apoptosis since this constitutes a common end point of epithelial and myeloid differentiation. Elucidation of Drg1 function, e.g. by transfection into undifferentiated colon epithelial and myeloid cell lines, will be the subject of further study. Immunohistochemical localization of Drg1 protein in different tissues may provide clues as to Drg1 function. The prognostic significance of the downregulation of Drg1 expression and the change in its subcellular localization observed in colon tumors (Van Belzen et al., 1997) will be assessed in a large series of colorectal adenomas and carcinomas.

Conclusion

Molecular markers of colon epithelial cell differentiation may provide prognostic factors with better stratification potential than the histological grading of differentiation. Several genes have been identified from which the expression is modulated during in vitro differentiation of colon epithelial cells, and in neoplastic as compared to normal cells, including Drg1, adenylosuccinate lyase, nucleophosmin, ATPase6, HLA class I, and α-tubulin. The number of genes known to be differentially expressed in colon cancer is increasing rapidly due to technical innovations, such as DNA microarray hybridization and SAGE.

To employ these molecular data in patient care, two complementary strategies may be followed. First, antibodies may be generated against the proteins encoded by differentially expressed genes. Protein expression as detected by immunohistochemistry may then be correlated with prognosis and with other prognostic factors such as Dukes' stage. However, recently it was observed that more than 500 genes are differentially expressed in gastrointestinal cancer relative to normal intestinal epithelium (Zhang et al., 1997). The large amount of work involved in the approach outlined above will require selection of gene products, e.g. based on their expression differences or biological function. A second strategy might be to quantitate the expression of thousands of genes within a single tumor by e.g. DNA microarray hybridization. If it turns out to be possible to correlate genome-scale gene expression data with tumor behavior in a meaningful way, this may revolutionize our understanding of the malignant phenotype and greatly improve patient diagnosis and prognosis.

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References

Arends J.W., Wiggers T., Thijs C.T., Verstijnen C., Swaen G.J. and Bosman F.T. (1984). The value of secretory component (SC) immunoreactivity in diagnosis and prognosis of colorectal carcinomas. Am. J. Clin. Pathol. 82, 267-274.

Augenlicht L.H. and Heerdt B.G. (1992). Modulation of gene expression as a biomarker in colon. J. Cell. Biochem. 16G, 151-157.

Bedi A., Pasricha P.J., Akhtar A.J., Barber J.P., Bedi G.C., Giardiello F.M., Zehnbauer B.A., Hamilton S.R. and Jones R.J. (1995). Inhibition of apoptosis during development of colorectal cancer. Cancer Res. 55, 1811-1816.

De Bruine A.P., Wiggers T., Beek C., Volovics A., von Meyenfeldt M., Arends J.W. and Bosman F.T. (1993). Endocrine cells in colorectal adenocarcinomas: incidence, hormone profile and prognostic relevance. Int. J. Cancer 54, 765-771.

DeRisi J.L., Iyer V.R. and Brown P.O. (1997). Exploring the metabolic and genetic control of gene expression on a genomic scale. Science 278, 680-686.

Donlon T., Wyman A.R., Mulholland J., Barker D., Bruns G., Latt S. and Botstein D. (1986). Alpha-satellite-like sequences at the centromere of chromosome #8. Am. J. Hum. Genet. 39, A196 (abstract).

Fantini J., Abadie B., Tirard A., Remy L., Ripert J.P., El Battari A. and Marvaldi J. (1986). Spontaneous and induced dome formation by two clonal cell populations derived from a human adenocarcinoma cell line, HT29. J. Cell Sci. 83, 235-249.

Ferrero S., Buffa R., Pruneri G., Siccardi A.G., Pelagi M., Lee A.K., Coggi G. and Bosari S. (1995). The prevalence and clinical significance of chromogranin A and secretogranin II immunoreactivity in colorectal adenocarcinomas. Virchows Arch. 426, 587-592.

Flohil C.C., Janssen P.A. and Bosman F.T. (1996). Expression of Bcl-2 protein in hyperplastic polyps, adenomas, and carcinomas of the colon. J. Pathol. 178, 393-397.

Gordon J.I. and Hermiston M.L. (1994). Differentiation and self-renewal in the mouse gastrointestinal epithelium. Curr. Opin. Cell Biol. 6, 795-803.

Grogan L., Behan, K.A. and Johnston P.G. (1996) Recent advances in the biology of colorectal cancer. Histol. Histopathol. 11, 807-820.

Habib N.A., Dawson P.M., Bradfield J.W., Williamson R.C. and Wood C.B. (1986). Sialomucins at resection margin and likelihood of recurrence in colorectal carcinoma. Br. Med. J. (Clin. Res. Ed.) 293, 521-523.

Hamada Y., Oishi A., Shoji T., Takada H., Yamamura M., Hioki K. and Yamamoto M. (1992). Endocrine cells and prognosis in patients with colorectal carcinoma. Cancer 69, 2641-2646.

Heerdt B.G., Houston M.A., Rediske J.J. and Augenlicht L.H. (1996). Steady-state levels of mitochondrial messenger RNA species characterize a predominant pathway culminating in apoptosis and shedding of HT29 human colonic carcinoma cells. Cell. Growth Diff. 7, 101-106.

Ho S.B. (1992). Cytoskeleton and other differentiation markers in the colon. J. Cell. Biochem. Suppl. 16G, 119-128.

Ho S.B., Itzkowitz S.H., Friera A.M., Jiang S.H. and Kim Y.S. (1989). Cell lineage markers in premalignant and malignant colonic mucosa. Gastroenterology 97, 392-404.

Hoog C. (1991). Isolation of a large number of novel mammalian genes by a differential cDNA library screening strategy. Nucl. Acids Res. 19, 6123-6127.

Huang S.W., Fogh J. and Hong R. (1976). Synthesis of secretory component by human colon cancer cells. Scand. J. Immunol. 5, 263-268.

Jackson R.C., Morris H.P. and Weber G. (1977). Enzymes of the purine ribonucleotide cycle in rat hepatomas and kidney tumors. Cancer Res. 37, 3057-3065.

Jansson D., Gould V.E., Gooch G.T., Rittenhouse H.G., Shin S.S., Manderino G.L., Tomita J.T. and Staren E.D. (1988). Immunohistochemical analysis of colon carcinomas applying exocrine and neuroendocrine markers. APMIS 96, 1129-1139.

Kikuchi Y., Dinjens W.N. and Bosman F.T. (1997). Proliferation and apoptosis in proliferative lesions of the colon and rectum. Virchows Arch. 431, 111-117.

Kokame K., Kato H. and Miyata T. (1996). Homocysteine-respondent genes in vascular endothelial cells identified by differential display analysis: GRP78/BiP and novel genes. J. Biol. Chem. 271, 29659-29665.

Kronborg O. (1993). Staging and surgery for colorectal cancer. Eur. J. Cancer. 29A, 575-583.

Lev R., Lance P. and Camara P. (1985). Histochemical and morphologic studies of mucosa bordering rectosigmoid carcinomas: comparisons with normal, diseased, and malignant colonic epithelium. Hum. Pathol. 16, 151-161.

Liang P. and Pardee A.B. (1992). Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257, 967-971.

Lichter P., Tang C.J., Call K., Hermanson G., Evans G.A., Housman D. and Ward D.C. (1990). High-resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. Science 247, 64-69.

Lin T.M. and Chang C. (1997). Cloning and characterization of TDD5, an androgen target gene that is differentially repressed by testosterone and dihydrotestosterone. Proc. Natl. Acad. Sci. USA 94, 4988-4993.

Moller P., Momburg F., Koretz K., Moldenhauer G., Herfarth C., Otto H.F., Hammerling G.J. and Schlag P. (1991). Influence of major histocompatibility complex class I and II antigens on survival in colorectal carcinoma. Cancer Res. 51, 729-736.

Nozawa Y., Van Belzen N., Van der Made A.C.J., Dinjens W.N.M. and Bosman F.T. (1996). Expression of nucleophosmin/B23 in normal and neoplastic colorectal mucosa. J. Pathol. 178, 48-52.

Ogata S., Uehara H., Chen A. and Itzkowitz S.H. (1992). Mucin gene expression in colonic tissues and cell lines. Cancer Res. 52, 5971-5978.

Podolsky D.K. (1993). Regulation of intestinal epithelial proliferation: a few answers, many questions. Am. J. Physiol. 264, G179-186.

Potten C.S., Wilson J.W. and Booth C. (1997). Regulation and significance of apoptosis in the stem cells of the gastrointestinal epithelium. Stem Cells. 15, 82-93.

Quaroni A., Nichols B.L., Quaroni E., Hurst K., Herrera L., Weiser M.M. and Hamilton S.R. (1992). Expression and different polarity of aminopeptidase N in normal human colonic mucosa and colonic tumors. Int. J. Cancer 51, 404-411.

Rabenandrasana C., Baghdiguian S., Roccabianca M., Brunet M., Marvaldi J. and Fantini J. (1990). The concentration of glucose in the culture medium determines the effect of suramin on the growth and differentiation of the human colonic adenocarcinoma cell clone HT29-D4. Cancer Lett. 53, 109-115.

Reed V.L., Mack D.O. and Smith L.D. (1987). Adenylosuccinate lyase as an indicator of breast and prostate malignancies: a preliminary report. Clin. Biochem. 20, 349-351.

Sagerstrom C.G., Sun B.I. and Sive H.L. (1997). Subtractive cloning: past, present, and future. Annu. Rev. Biochem. 66, 751-783.

Schena M., Shalon D., Davis R.W. and Brown P.O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270, 467-470.

Taguchi T., Kijima H., Mitomi T. and Osamura R.Y. (1991). Immunohistochemical study of colorectal adenocarcinomas and adenomas with antibodies against carcinoembryonic antigen (CEA), CA19-9, keratin, alpha-tubulin and secretory component (SC). Gastroenterol. Jpn. 26, 294-302.

Ten Kate J., Wijnen J.T., Boldewijn J., Khan P.M. and Bosman F.T. (1985). Immunohistochemical localization of adenosine deaminase complexing protein in intestinal mucosa and in colorectal adenocarcinoma as a marker for tumour cell heterogeneity. Histochem. J. 17, 23-31.

Thompson J.D., Higgins, D.G. and Gibson T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucl. Acids Res. 22, 4673-4680.

Van Belzen N., Diesveld M.P.G., Van der Made A.C.J., Nozawa Y., Dinjens W.N.M., Vlietstra R., Trapman J. and Bosman F.T. (1995). Identification of mRNAs that show modulated expression during colon carcinoma cell differentiation. Eur. J. Biochem. 234, 843-848.

Van Belzen N., Dinjens W.N.M., Diesveld M.P., Groen N.A., van der Made A.C., Nozawa Y., Vlietstra R., Trapman J. and Bosman F.T. (1997). A novel gene which is up-regulated during colon epithelial cell differentiation and down-regulated in colorectal neoplasms. Lab. Invest. 77, 85-92.

Van der Wurff A.A.M., Ten Kate J., Marx P.T.J., Van der Linden E.P.M., Beek C.C.L., Bovelander F.-J., Dekker J., Dinjens W.N.M., Von Meyenfeldt M.F., Arends J.-W. and Bosman F.T. (1998). Expression of a marker for colonic crypt base cells is correlated with poor prognosis in human colorectal cancer. Gut 42, 63-70.

Velculescu V.E., Zhang L., Vogelstein B. and Kinzler K.W. (1995). Serial analysis of gene expression. Science 270, 484-487.

Welsh J., Chada K., Dalal S.S., Cheng R., Ralph D. and McClelland M. (1992). Arbitrarily primed PCR fingerprinting of RNA. Nucl. Acids Res. 20, 4965-4970.

Wiegant J., Ried T., Nederlof P.M., van der Ploeg M., Tanke H.J. and Raap A.K. (1991). In situ hybridization with fluoresceinated DNA. Nucl. Acids Res. 19, 3237-3241.

Wilson R., Ainscough R., Anderson K., Baynes C., Berks M., Bonfield J., Burton J., Connell M., Copsey T., Cooper J., Coulson A., Craxton M., Dear S., Du Z., Durbin R., Favello A., Fraser A., Fulton L., Gardner A., Green P., Hawkins T., Hillier L., Jier M., Johnston L., Jones M., Kershaw J., Kirsten J., Laisster N., Latreille P., Lightning J., Lloyd C., Mortimore B., O'Callaghan M., Parsons J., Percy C., Rifken L., Roopra A., Saunders D., Shownkeen R., Sims M., Samldon N., Smith A., Smith M., Sonnhammer E., Staden R., Sulston J., Thierry-Mieg J., Thomas K., Vaudin M., Vaughan K., Waterston R., Watson A., Welnstock L., Wilkinson-Sproat J. and Wohldman P. (1994). 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. Nature 368, 32-38.

Wiltz O., Steele G.D. Jr. and Mercurio A.M. (1991). Expression of enzymatically active sucrase-isomaltase is a ubiquitous property of colon adenocarcinomas. Gastroenterology 100, 1266-1278.

White J.A., McAlpine P.J., Antonarakis S., Cann H., Eppig J., Frazer K., Frezal J., Lancet D., Nahmias J., Pearson P., Peters J., Scott A., Scott H., Spurr N., Talbot Jr. C. and Povey S. (1997). Guidelines for human gene nomenclature. Genomics 45, 468-471.

Zhang L., Zhou W., Velculescu V.E., Kern S.E., Hruban R.H., Hamilton S.R., Vogelstein B. and Kinzler K.W. (1997). Gene expression profiles in normal and cancer cells. Science 276, 1268-1272.

Zweibaum A., Laburthe M., Grasset E. and Louvard D. (1991). Use of cultured cell lines in studies of intestinal cell differentiation and function. In: Handbook of physiology. Schultz S.G. (ed). American Physiological Society. Bethesda. pp 223-255.