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Original contribution

The insulin-like growth factor type 1 receptor and colorectal neoplasia: insights into invasion ☆

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Summary This study examines the expression of the insulin-like growth factor type 1 receptor (IGF-1R) in colorectal neoplasia. Previous studies have shown that the IGF-1R is expressed at high levels in normal embryonic stem cells and in many cancer phenotypes. However, lower IGF-1R levels are expressed in some advanced cancer phenotypes. The timing of and the reasons for these changes in expression during the evolution of a cancer are not understood. Here, we examine IGF-1R expression in the evolution of colorectal cancer by means of Northern blotting and immunohistochemistry validated by tissue and reagent controls and Western blotting. We show for the first time that (1) in normal colorectal crypts, epithelial stem cells in the basal crypt region express high IGF-1R levels, which decrease to low levels when these cells migrate to and differentiate in the mid and upper crypt regions; (2) in tumor initiation in aberrant crypt foci, all of the transformed cells express high levels of the IGF-1R at stem cell levels throughout the crypt axis; (3) in tumor progression in adenomatous and cancerous crypts, tumor cells of an epithelial type morphology express high levels of the IGF-1R; (4) in advanced cancers, low levels of the IGF-1R are expressed in invasive foci where cancer cells dedifferentiate to a mesenchymal-type morphology and show a loss of cell adhesion. Interestingly, these cells can form an alternating pattern with mesenchymal type cells that show cell adhesion and high levels of IGF-1R expression. In summary, this study shows that high-level IGF-1R expression in colorectal neoplasia is initiated by an abnormality of stem cell programed differentiation in the aberrant crypt focus. However, low-level IGF-1R expression is found in some invasive cancers where it is consequent to cancer cell dedifferentiation to a mesenchymal type morphology with loss of cell adhesion.

1. Introduction

The insulin-like growth factor type 1 receptor (IGF-1R) is a highly conserved transmembrane receptor tyrosine kinase that is highly expressed in embryonic stem cells but is expressed at low levels in most adult differentiated tissues [1-3]. In contrast, most cancer phenotypes including cancers

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of the breast [4], prostate [5], colon [6-8], pancreas [9], liver [10], ovary [11], bladder [12], and brain [13] highly express the IGF-1R. However, some recent studies have shown that some of the more advanced cancer phenotypes of the colorectum [14], breast [15], and prostate [16] that show metastases can also show comparatively lower levels of the IGF-1R. The timing of and the possible reasons for these differences in gene expression during tumor initiation or progression remain unclear and are examined in this study.

The IGF-1R has a highly regulated promoter exhibiting high intrinsic basal activity [17]. The promoter is GC-rich and TATA-less with a single initiator. Cis-elements acting via mostly Sp1 sites in the proximal 5′ -flanking region in concert with inhibition from trans regulators such as WT-1, p53, and myc acting on the 5′ -flank and 5′ -UTR are thought to be important in determining the low transcript levels seen in most adult differentiated tissues [18-20]. Indeed, these tumor suppressors have been cited as putative mechanisms for the rebound in IGF-1R expression seen in the tumor cell. However, the mechanisms of IGF-1R promoter control during tumor initiation or progression remain entirely speculative at this stage.

The IGF-1R coregulates through multiple signaling networks diverse cell behaviors that include proliferation, differentiation, survival, and adhesion (reviewed by Le Roith [21], Mauro and Surmacz [22], and Valentinis and Baserga [23]). Components of the signaling networks that include Ras/Raf/MAPK, PI3K/Akt/Bcl-2/BAD, β-catenin/E-cadherin/APC are targeted by mutational or expressive change in most human cancers [24] and hence are thought to confer the altered cell behaviors of the tumor phenotype. Early studies showed that functionality of the IGF-1R was a prerequisite for neoplastic transformation and for survival of the transformed cell in vitro and in vivo (reviewed by Baserga [25]). More recent evidence has pointed to putative roles for the IGF-1R in cell adhesion and in cancer cell dedifferentiation [22,26,27]. The IGF-1R, via its signaling moiety IRS-1, is thought to translocate as a tripartite complex with E-cadherin and β-catenin to coregulate cell adhesion-dependent gene transcription in a poorly understood manner [26,27]. Whether any of these mostly in vitro functions of the IGF-1R have any correlates with cell behaviors in human cancer tissue is unknown.

The colorectal normal crypt-ACF-polyp-cancer sequence provides a valuable model system to examine gene expression profiles during the processes of normal cellular differentiation, tumor initiation, and tumor progression. The cell compartmentalization of the normal colorectal crypt has been described in detail and consists of the basal crypt stem cell region, the mid crypt transit amplifying (or proliferating and differentiating) region, and the upper crypt terminally differentiated region [28]. Gene expression profiles can thus be examined during the process of normal differentiation in normal colorectal crypts. These can be compared to the gene expression profiles seen during tumor initiation in ACFs and during tumor progression in adenomatous and cancerous crypts.

In advanced cancers, dedifferentiation, invasion, and metastasis occur, but the morphogenesis and gene expression changes that underlie these processes are poorly understood. Cancer cell dedifferentiation has been compared with embryologic epithelial mesenchymal transformation (EMT), but this is a conceptually different process. Furthermore, there are practical difficulties in inferring the temporal and morphological sequence of events that underlie these processes in heterogeneous cancer specimens.

The primary aim of this study was to examine the expression profile of the IGF-1R in the colorectal normal crypt-ACF-polyp-cancer sequence to discern the timing and nature of any change in IGF-1R expression during this sequence. In addition, the expression profile of the IGF-1R was examined in relation to the expressions of the WT-1 and c-myc regulators as well as in relation to cell morphologies in normal and tumoral crypts. The study's secondary aim was therefore to discern possible reasons for expression change in the IGF-1R during tumor initiation and progression along with the possible reasons for the low levels of IGF-1R expression seen in advanced cancers in some previous studies.

2. Materials and methods

2.1. Tissue samples and demographics

Tissue samples were sourced from patients undergoing elective and emergency colorectal resection for benign and malignant conditions at the Colorectal Unit, Western General Hospital, Edinburgh, UK. A total of 8 fresh and 69 archival resection specimens were examined by means of Northern blotting, immunohistochemistry, or Western blotting. Patient demographics showed a male/female ratio of 1.1:1.0 and a mean age of 72.0. There were 6 well-differentiated, 7 moderately differentiated, and 8 poorly differentiated cancers showing stages pT2N0 or less (2 specimens), pT3N0 (9 specimens), and pT3N1 or greater (10 specimens). Fresh specimens were rapidly processed on ice, microdissecting normal mucosa off the underlying muscularis, and dissecting tumoral mucosa from the lumenal aspect of the specimen to avoid interfering with disease staging. Matched normal mucosa was taken from either the proximal or distal resection margin, whichever was furthest from the tumoral specimen to avoid interfering with disease staging. Polyps were sourced from polypectomies, cancer resections, and benign resections. Fresh specimens were submitted for Northern blotting, immunohistochemistry, and Western blotting. Paraffin-embedded archival specimens were taken either concurrently at the time of taking the fresh specimens or from 61 recent resections (within 2 weeks) identified during the study period. Archival specimens were examined as per normal histologic staging, immunostaining representative sections widely from the body of the tumor, its distal and circumferential margins, and from any obviously invasive margin. In addition,