ECM-related Genes in Colorectal Carcinoma

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During bowel cancer screening programs, many diagnostically problematic polyps are removed. The greatest challenge is to distinguish between adenomas with epithelial misplacement and adenomas with early carcinoma, considering the diagnosis affects prognosis and treatment. Researcher's aim was to analyze the expression of extracellular matrix related genes and proteins, namely DCN, EPHA4, FN1, SPARC, SPON2, and SPP1. Differences were observed in most of the analyzed genes and proteins in adenoma with epithelial misplacement in comparison to adenoma with early carcinoma, reflecting inflammatory stromal reaction to traumatisation and misplacement of dysplastic glands in the submucosa in the former, and desmoplastic stromal reaction to true invasion of dysplastic glands in the submucosa in the latter.

Keywords: extracellular matrix,colorectal carcinoma ; ,adenoma with epithelial misplacement

1. Introduction

Colorectal carcinoma (CRC) is a heterogeneous disease, which usually evolves gradually, forming a spectrum of lesions, due to accumulation of genetic mutations and epigenetic alterations in key growth regulatory and differentiation genes[1][2][3][4]. The correct histopathologic diagnosis of different stages of CRC is of vital importance enabling ¸to choose the optimal treatment. Endoscopic removal is the treatment of choice for adenomas and adenomas with epithelial misplacement (AEM), also referred to as pseudoinvasion, since these lesions do not metastasize and additional surgical treatment is not necessary. In contrast, adenomas with early carcinoma (AEC) are capable of metastasizing and in some patients, surgical removal of the affected bowel with regional lymph nodes is needed.

CRC screening programs worldwide have enabled to detect and remove a large number of early polypoid lesions, including adenomas, AEM and AEC[5]. In the majority of cases, histopathology examination is straightforward, but there is a growing number of cases with ambiguous histopathologic features. For this reason, researchers would need additional histopathologic, immunohistochemical and/or genetic markers to be used in problematic lesions[6][7]. The most challenging task is to distinguish between AEM and AEC. In both lesions, dysplastic glands are found in the submucosa, but only in AEC, it is the result of true invasion[7][8]. In AEM, dysplastic glands are present in the submucosa due to traumatization and consequent reparation. This is typically a result of intraluminal traumatic injury of the larger polyps due to combination of different factors (narrow, highly motile sigmoid colon, solid fecal material and diverticulosis)[6]. Histologically, true invasion is characterized by severe dysplasia and desmoplastic stromal reaction while in epithelial misplacement, dysplastic glands in the submucosa appear similar to the surface of adenoma and are usually accompanied by lamina propria[7]. Its characteristic features are also hemosiderin depositions and mucus lakes[7].

Markers to distinguish between AEM and AEC are lacking. Recently, microRNAs have been associated with the development of cancer, as they influence the expression of their regulated gene(s)[9]. In CRC, it has been shown that microRNA expression profile differs among the adenoma-carcinoma sequence[10][11]. Researchers hypothesized that in terms of gene expression, AEM is similar to adenoma, and AEC is similar to CRC. In previous study[5], we used a bioinformatics approach to identify candidate genes for biomarkers that would distinguish between adenoma and CRC. In this study, we analyzed the expression of extracellular matrix (ECM) related genes decorin (*DCN*), erythropoietin-producing hepatoma receptor A4 (*EPHA4*), fibronectin 1 (*FN1*), secreted protein acidic and cysteine rich (*SPARC*), spondin 2 (*SPON2*) and secreted phosphoprotein 1 (*SPP1*) in AEM in comparison to AEC.

2. Extracellular Matrix-Related Genes and Their Regulatory microRNAs in Problematic Colorectal Polyps

the expression of selected ECM-related genes (*DCN*, *EPHA4*, *FN1*, *SPARC*, *SPON2*, and *SPP1*) was similar in adenoma and AEM but differed from the expression in AEC and advanced carcinoma[2]. Researchers found two genes, *DCN* and *SPP1*, showing different expression in AEM compared to either AEC or advanced carcinoma. Moreover, the

expression of their regulatory microRNAs was significantly negatively (*hsa-miR-200c* for *DCN* and *hsa-miR-146a* for *SPP1*) or positively (*hsa-let-7a* for *EPHA4*) associated with the expression of their regulated gene.

Moreover, *SPON2* and *SPARC* showed up-regulation in AEC and advanced carcinoma, and down-regulation in adenoma and AEM. This expression pattern confirms researcher's hypothesis that the expression of selected ECM-related genes is similar in adenoma and AEM, but differs from the expression in AEC and advanced carcinoma. Although the expression of one of the regulatory microRNAs for *SPARC* was inversely proportional to the expression of *SPARC*, statistical analysis did not show any significant correlation. Unfortunately, the expression pattern of other selected regulatory microRNAs for *SPARC* and *SPON2* was neither inverse nor statistically significantly correlated with their regulated gene.

Surprisingly, expression of gene *EPHA4* was highly up-regulated in AEM, where dysplastic glands are present in the submucosa due to traumatization and consequent reparation, in comparison to other lesions. Although its expression pattern did not confirm researcher's hypothesis, its high expression indicates that *EPHA4* might serve as one of the markers of epithelial misplacement. So far, expression of *EPHA4* and of some other receptors of EPH family has been used do differentiate between various stages of non-small cell lung carcinoma. Additionally, their high expression correlated with low stage and presence of inflammation[12][13].

The remaining gene, *FN1*, did not show any pattern that would either confirm or reject researcher's hypothesis. However, on the protein level, FN1 showed discrete differences between AEM and AEC in comparison to adenoma. Fibronectin promotes fibroblast migration directly, and promotes proliferation by regulating the bioavailability of TGFβ. Fibronectin also binds to TNFα, which promotes chemotaxis and expression of matrix metalloproteinase 9 in monocytes[14]. TNF in turn has an effect on the up-regulation of SPP1[15][16].

ECM is formed by a diverse spectrum of molecules including proteins, proteoglycans, glycoproteins, and polysaccharides[17][18]. They provide unique biochemical, biophysical and biomechanical properties[19]. Under pathological conditions, the dynamics of the ECM changes. The main contributors of ECM remodeling are matrix metalloproteinases[17]. Study by Li et al. showed that expression of matrix metalloproteinases 2 and 9 increased in CRC compared to healthy colon mucosa[20]. Combination of functional domains, characteristic for matrix metalloproteinases, allows affecting several cellular processes, such as proliferation and apoptosis. Different experiments showed that degradation of surrounding tissue by matrix metalloproteinases have several functions that favor tumor progression by modulation of growth factors, inflammatory proteins, membrane receptors, adhesion molecules, and chemoattractants[21].

Inflammation is one of the pathological conditions that cause aberrant expression of ECM components. In inflamed tissues, cytokines e.g., TGFβ, TNF, and IFNγ cause protease secretion and initiate a cycle of ECM degradation and synthesis[15]. Consequently, proteases generate different chemotactic fragments that in turn recruit different immune cells to the site of the inflammation[14]. Collagen degradation products act as chemoattractant for neutrophil recruitment[17]. Therefore, the remodeled ECM of inflamed tissues affects the propagation of the inflammatory response and the development of the chronicity[15]. Tissues that are subject to chronic inflammation generally exhibit high cancer incidence[22].

In cancer, ECM becomes more stiff and rigid, which is among other the consequence of aberrant collagen and fibronectin deposition as well as excessive crosslinking by lysyl oxidases, as a result of desmoplasia[23]. Changes in the ECM stiffness causes the surrounding tissue to exhibit different biomechanical and biophysical properties, which in turn have, for example an effect on TGFβ signaling. Moreover, increase in collagen deposition up-regulates integrin signaling and can thus promote survival and proliferation. Therefore, the emerging environmental signals stimulate proliferative and apoptotic mechanisms, which are thought to lead to the selection of apoptosis-resistant cells with enhanced invasive potential[24]. The main contributors of altered activities of the ECM remodeling enzymes and ECM metabolism are stromal cells, including cancer-associated fibroblasts and immune cells. In advanced stages of cancer development, other cell types may also contribute to the altered composition of the ECM[18]. TGFβ is one of the essential cytokines that activate the fibrotic response and cancer stroma. TGFβ promotes myofibroblast differentiation and the recruitment of immune cells, inhibiting the anti-tumor immune responses and affecting epithelial and endothelial cell differentiation by controlling several different functions in most of the cells that form fibrous tissue[25].

The bioavailability and the downstream effects of TGFβ are lessened by binding of DCN to TGFβ reducing fibrous tissue[26][27]. Moreover, DCN might be one of the regulators of the synthesis of the ECM components and expression of collagenase, inhibitor of collagen I maturation which contributes to angiogenesis in the tumor[27]. TGFβ was also shown to stimulate SPARC function as an essential factor in tumor cell migration[28] where it participates as one of the regulators of the fibronectin network assembly. Otherwise, SPARC also participates to angiogenesis and wound healing[29]. A role in the immune response was also reported for SPON2 and SPP1. SPON2 participates in activation of immune response and recruitment of inflammatory cells[30], whereas SPP1 along with other pro-inflammatory factors contributes to tumor

growth[31], angiogenesis by stimulating VEGF and macrophage recruitment[32]. SPP1 may be also involved in malignant transformation by transactivating different transcription factors. Moreover, SPP1 is one of the proteins needed for the process of fibroblast to myofibroblast differentiation[33].

Cell adhesion molecules on the endothelial cell surface interact with components of the ECM, such as fibronectin, collagens, and laminin to regulate both the recruitment of circulating leukocytes and modulate intracellular signaling pathways, which control endothelial permeability[14]. The EPH receptors are tyrosine kinase cell surface receptors that bind to their membrane bound ligands, ephrins, and modulate vascular permeability during inflammation[34]. It has been shown that up-regulated expression of *EPHA4* contributes to the spinal cord scar formation, since spinal cord injury in mice lacking expression of *EPHA4* resulted in axonal regeneration[34][35]. According to Ivanov et al., *EPHA4* receptor is mainly expressed in lymphocytes, monocytes, granulocytes and dendritic cells[36] and participates in regulation of T-cell development[34] and mediates T-cell chemotaxis[37]. Namely mice with *EPHA4* knockdown exhibited a blockage in T-cell maturation[34].

Researcher's results indicate that some ECM-related genes could be post-transcriptionally regulated since they showed inverse correlation with their regulatory microRNA, e.g., *SPP1* and hsa-*miR-146a*[38] and *DCN* and *hsa-miR-200c*. Additionally, some microRNAs might be useful for distinguishing AEM from AEC, e.g., *hsa-miR-29c*. As with researcher's bioinformatics approach, comparing adenoma to carcinoma to identify candidate genes to be used as markers for true invasion, bioinformatics analysis for identification of microRNAs to be used as markers would be also interesting. This approach was recently used by different research groups[9][10][11]. The true markers should be independent of specimens used[9][10]. Furthermore, inverse correlation between identified microRNAs and mRNAs would give us deeper understanding of the mechanisms of true invasion and epithelial misplacement.

The most important limitations of our study are the lack of functional validation of the analyzed microRNAs and a relatively small number of patients. The latter is due to the fact that our study included formalin-fixed paraffin-embedded (FFPE) tissue samples. In FFPE tissue, nucleic acids are fragmented and therefore difficult to analyze, but a great advantage of FFPE tissue is that samples are first evaluated by pathologists, enabling appropriate diagnosis. In our study, only samples that had successfully passed initial quality control and samples with stable expression of the reference genes were selected for further analysis, thus limiting the number of included samples. Furthermore, our study focused on problematic polyps, i.e., those containing either epithelial misplacement or early cancer, which usually occupy a small area, enabling a limited amount of appropriate tissue for analyses. For all these reasons, researcher's results must be interpreted with caution.

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