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Original contribution

Prognostic significance of stromal GREM1 expression in colorectal cancer☆,☆☆

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Summary Cancer-associated fibroblasts are the dominant cell population in the cancer stroma. Gremlin 1 (GREM1), an antagonist of the bone morphogenetic protein pathway, is expressed by cancer-associated fibroblasts in a variety of human cancers. However, its biological significance for cancer patients is largely unknown. We applied RNA in situ hybridization to evaluate the prognostic value of stromal GREM1 expression in a large cohort of 670 colorectal cancers (CRCs). Overall, GREM1 expression in CRCs was lower than that of the matched normal mucosa, and GREM1 expression had a strong positive correlation with BMI1 and inverse correlations with EPHB2 and OLFM4. RNA in situ hybridization localized the GREM expression to smooth muscle cells of the muscularis mucosa and fibroblasts around crypt bases and in the submucosal space of a normal colon. In various colon polyps, epithelial GREM1 expression was exclusively observed in traditional serrated adenomas. In total, 44% of CRCs were positive for stromal GREM1, which was associated with decreased lymphovascular invasion, a lower cancer stage, and nuclear β-catenin staining. Stromal GREM1 was significantly associated with improved recurrence-free and overall survival, although it was not found to be an independent prognostic marker in multivariate analyses. In addition, for locally advanced stage II and III CRC, it was associated with better, stage-independent clinical outcomes. In summary, CRCs are frequently accompanied by GERM1-expressing fibroblasts, which are closely associated with low lymphovascular invasion and a better prognosis, suggesting stromal GREM1 as a potential biomarker and possible candidate for targeted therapy in the treatment of CRCs.

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1. Introduction

Cancer is a complex mixture of malignant cells and surrounding tumor stroma, including fibroblasts, infiltrating immune cells, blood and lymphatic endothelial cells, and extracellular matrix [1]. Among these cell types, cancer-associated fibroblasts (CAFs) are known to actively interact with cancer cells and promote cancer cell proliferation, angiogenesis, invasion, and metastasis [2]. It has been demonstrated that tumors formed in mice after transplanting cancer cells admixed with CAFs are more malignant than those formed by transplanting cancer cells alone or cancer cells with normal fibroblasts [3,4]. Therefore, molecules enriched in CAFs such as the FAP, CXCL12, HGF, and cathepsin K are promising selective therapeutic targets [5], and several clinical studies targeting CAFs in human cancers have been proposed [6-8].

Gremlin 1 (GREM1) is a member of the bone morphogenetic protein (BMP) antagonists, and it plays a critical role in embryonic and postnatal development, as well as in fibrotic diseases such as diabetic nephropathy [9] and pulmonary hypertension [10]. GREM1 also exerts proangiogenic activity in the endothelium of human lung tissues [11] and is a key regulator of synoviocyte hyperplasia and invasiveness [12]. In normal colon crypts, GREM1 was found to be expressed by intestinal pericryptal fibroblasts and smooth muscle cells of muscularis mucosa (MM), creating an increasing gradient toward the crypt [13]. This gradient contributes to maintaining the intestinal stem cell niche in the colonic basal crypt region [13]. Sneddon et al [14] reported wide expression of GREM1 in the CAFs of basal cell carcinoma, which promoted proliferation of cultured basal cell carcinoma cells by inhibiting the BMP signaling pathway. They further found that GREM1 is expressed by stromal cells in diverse human carcinomas, including colon cancer [3]. Therefore, GREM1 expression in CAFs may influence colorectal cancer (CRC) progression. However, only a few studies have investigated the potential prognostic value of GREM1 expression in CRC [15,16].

Several reports have attempted to localize GREM1 expression in CRCs. RNA in situ hybridization (ISH) showed specific and clear visualization of GREM1 messenger RNA (mRNA) in human formalin-fixed, paraffin-embedded (FFPE) specimens [13-15]. By contrast, immunohistochemistry with anti-GREM1 antibodies consistently produced nonspecific staining in epithelial cells and did not show distinct GREM1 expression in the smooth muscle cells of MM and fibroblasts near crypt bases in normal colonic mucosa [16-18]. Furthermore, for a secreted protein, RNA ISH has been suggested as a better tool for determining the cellular origin of protein within tissue [19]. Therefore, in this study, we applied RNA ISH for GREM1 to investigate the stromal GREM1 expression in a large cohort of CRC patients and analyzed the prognostic significance of GREM1, as well as its correlation with clinicopathological characteristics.

2. Materials and methods

2.1. Patients

This study was approved by the institutional review board of Seoul National University Hospital and Jeju National University Hospital. CRC samples were collected from 1133 patients who underwent surgical resection at Seoul National University Hospital, Seoul, Korea, from January to December 2006. Exclusion criteria include refusal of molecular study, noninvasive cancers, neoadjuvant treatment history, familial adenomatous polyposis, and multiple or recurrent tumors. Clinicopathological data, including the patient age, sex, tumor size, location, histologic type, evidence of lymphovascular invasion, and TNM pathological stage, were obtained by reviewing the pathological reports. In addition, molecular data including *KRAS*/*BRAF* mutation, microsatellite instability status, and CpG island methylator phenotype were obtained from previous studies [20,21]. Patient outcomes included information on the following: recurrence, survival, and follow-up time. Benign colon polyps including hyperplastic polyps (n = 3), sessile serrated adenomas (n = 12), traditional serrated adenomas (TSAs; n = 13), and tubular adenomas with low-grade dysplasia (n = 13) were obtained from endoscopic polypectomies at Seoul National University hospital. In addition, 32-paired, fresh-frozen CRC tissues and matched noncancerous tissues were provided by the Jeju National University Hospital Biobank, a member of the National Biobank of Korea.

2.2. Tissue microarray construction

Twenty tissue microarrays (TMAs) containing 1133 CRCs and 2 TMAs containing 41 colon polyps were generated. In brief, through histologic examination, the representative tumor portions in which tumor cells comprise more than 70% of cell populations were marked in each case. Tumor core of 2-mm diameter was obtained from the corresponding area of FFPE tissue block and arranged in a new recipient paraffin block using a trephine apparatus (SuperBioChips Laboratories, Seoul, Korea).

2.3. Immunohistochemistry

Immunohistochemistry was performed on 4-μm TMA sections using a BOND-MAX automated immunostainer and Bond Polymer Refine Detection kit (Leica Microsystems, Wetzlar, Germany) according to the manufacturer's instructions. The primary antibody included an anti–β-catenin (Novocastra Laboratories, Newcastle, United Kingdom; 17C2; 1:800). β-Catenin staining was considered as positive when more than 10% of the tumor cell nuclei were strongly stained for β-catenin.