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KRAS and *PIK3CA* mutations in colorectal adenocarcinomas correlate with aggressive histological features and behavior☆,☆☆

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Summary Tumor budding (TB) in colorectal carcinoma (CRC) is related to epithelial-mesenchymal transition and has been recently characterized as an indicator of poor prognosis along with lymphovascular tumor emboli, perineural invasion, and an infiltrative growth pattern. Mutations in the genes of the Ras–mitogen-activated protein kinase and phosphatidylinositol-4,5-bisphosphate 3-kinase pathways are associated with epithelial-mesenchymal transition and an aggressive CRC phenotype and have been used in patient stratification for anti–epidermal growth factor receptor therapies; however, the impact of these mutations on CRC morphology and behavior remains unclear. In this study, using a multigene panel, we detected *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *TP53*, and *POLE* mutations in 90 CRCs and investigated their associations with clinicopathological parameters, including TB. Our results showed that 21 of 34 tumors with high-grade TB had *KRAS* mutations ($P = .001$) and *KRAS* G12D and *PIK3CA* exon 9 variants were significantly associated with high-grade TB ($P = .002$ and .006, respectively); furthermore, tumors with *KRAS* mutations in exons 3 and 4 tended to have lymphovascular tumor emboli and perineural invasion ($P = .044$ and .049, respectively). *PIK3CA* exon 9 mutations indicated a tendency for shorter disease-free survival ($P = .030$), whereas *BRAF* mutations were associated with extracellular mucin deposition ($P = .016$). Our study revealed a correlation of *KRAS* mutations with high-grade TB, an association of certain *KRAS* and *PIK3CA*

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variants with aggressive clinicopathological features, as well as a possible relationship between *BRAF* mutations and mucin production in CRC.

1. Introduction

In recent years, advances in the understanding of the molecular biology of colorectal carcinoma (CRC) have led to new molecular classifications of this disease based on genotype–phenotype correlations [1]. It has been established that small GTPase Ras/mitogen-activated protein kinase (MAPK) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathways, downstream pathways of the epidermal growth factor receptor (EGFR), are deregulated in CRC, yet it is still unclear how mutations in the genes encoding key signaling proteins of these pathways affect tumor histopathology and morphology.

Tumor budding (TB) is a notable histological feature of CRC, which has consistently been associated with adverse outcomes along with lymphovascular tumor emboli (LVE), perineural invasion (PNI), and an infiltrative growth pattern; however, it has become a focus of pathological analysis only in the last decade [2]. Most studies define *TB* as single cells or small cell clusters of fewer than 5 cells lacking glandular lumen formation, usually at the invasive front of a tumor. TB has been linked to epithelial-mesenchymal transition (EMT), and it has been shown that mutations activating the Ras-MAPK and PI3K pathways play critical roles in EMT and tumor progression [3,4]. Accordingly, it could be hypothesized that there is a correlation between histological signs indicating an aggressive cancer phenotype, such as TB, and genes in the Ras-MAPK and PI3K pathways.

Mutations in codons 12 and 13 in the *KRAS* gene encoding K-Ras GTPase confer resistance to anti-EGFR therapies in patients with metastatic CRC, and screening for these genetic variations has become standard in patient stratification for treatment. Furthermore, recent data indicate that mutations in different *KRAS* codons and in another Ras gene, *NRAS*, as well as in *BRAF* and *PIK3CA* genes encoding protein kinases of the Ras-MAPK and PI3K pathways, respectively, may predict poor therapeutic responses to anti-EGFR therapy [5-9]. The clinical value of these newly discovered mutations that have become an important part of CRC evaluation owing to advances in sequencing technologies underscores the necessity to clearly determine the impact of genetic variations in the Ras-MAPK and PI3K pathways on CRC morphology and behavior.

In this study, we performed a high-throughput mutation analysis of CRC cases to investigate the association between *KRAS, NRAS, BRAF,* and *PIK3CA* mutational profiles and clinicopathological parameters, including TB grade, LVE, PNI, tumor growth pattern, mucin production, and patient survival.

2. Materials and methods

2.1. Patient selection and histopathological review

A total of 104 patients who underwent standard radical colorectal surgery and regional lymphadenectomy for primary colorectal adenocarcinoma at Kangnam Sacred Heart Hospital in Seoul between January 2011 and December 2012 were retrospectively selected. Patients subjected to preoperative neoadjuvant chemoradiotherapy and those who did not sign a consent form were excluded. As a result, 90 patients were found eligible for the study. Demographic, clinical, and follow-up data were extracted from the patients' medical records. This study was approved by the Institutional Review Board committee of Kangnam Sacred Heart Hospital (IRB no. 2015-11-136).

Hematoxylin-eosin (H&E)–stained tumor tissue sections were reviewed by 2 pathologists (J. W. K. and M. E. H.) blinded to patient's clinical information. Tumors were classified according to the seventh edition of the American Joint Commission on Cancer staging system [10]. For each case, TB was graded based on the number of tumor buds as previously described by Ueno et al [11] with later modifications [2]. In brief, each slide was scanned at low-power magnification to identify areas with the highest budding density. After choosing 1 field with the densest budding, budding clusters/single cells were counted using a 20× objective lens (0.785 mm^2), and the specimens were graded according to TB: low grade (0-9 counts) and high grade (≥10 counts) (Fig. 1). Other histopathological parameters including growth pattern, LVE, PNI, and mucin production were also assessed.

2.2. DNA extraction

Formalin-fixed paraffin-embedded (FFPE) blocks that included the region with the highest TB and an adequate amount of tumor tissue were selected. Depending on tumor size assessed by histology, 5-10 sections of 10-μm thickness for each FFPE block were used for genomic DNA extraction. After deparaffinization with xylene and ethanol, DNA was purified using a QIAamp DNA kit for FFPE tissues (#56404; Qiagen, Hilden, Germany) according to the manufacturer's instructions.

2.3. Detection of *KRAS, NRAS, BRAF, PIK3, TP53, and POLE* mutations

Mutations were identified using the OncoMap_C1 panel with the Sequenom MassARRAY iPLEX-Pro platform (Sequenom, San Diego, CA) following the manufacturer's