Molecular pathology selected abstracts

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Findings of a genomewide analysis of people with irritable bowel syndrome

January 2022—Irritable bowel syndrome is the most common gastrointestinal disorder, affecting 10 to 15 percent of the world population, yet its etiology is unknown. Patients experience a spectrum of gastrointestinal symptoms, including diarrhea, constipation, abdominal cramping, and pain. Diet and behavior modification are the mainstays of treatment. Medications, including anticholinergics, tricyclic antidepressants, selective serotonin reuptake inhibitor antidepressants, and pain medications acting on the brain-gut neurocircuitry, may be prescribed. Given the disease's unclear etiology, patients often undergo extensive medical evaluation with negative results. The authors of this study used population data from the UK Biobank, in the United Kingdom, to perform a genetic meta-analysis of single nucleotide polymorphisms (SNPs) associated with patients of European ancestry with IBS. They created a digestive health questionnaire to inquire about IBS symptoms, severity, diagnoses, environmental exposures, and associated conditions, such as anxiety and depression. The authors emailed the questionnaire to UK Biobank participants and received a response rate of approximately 50 percent. Of the survey respondents who met the diagnostic criteria for IBS, only one-third had a hospital-documented diagnosis of IBS, suggesting a high prevalence of IBS underdiagnosis. Consistent with the epidemiology of IBS, females were more commonly affected than males, and a family history was more common in affected patients. Notably, participants reported increased rates of comorbidities, including atopic disease, anxiety, depression, history of abdominal surgeries (appendectomy, cholecystectomy, and hysterectomy), and increased childhood exposure to antibiotics, as compared to control subjects. In a genomewide association study, six IBS-associated loci were identified in 53,400 people with IBS through a discovery cohort using SNP data from the UK Biobank and Bellygenes initiatives and confirmed in an independent cohort from 23andMe. All of the loci were found in autosomes, and three of the six loci had previous associations with mood and anxiety disorders. Genetic fine-mapping to identify plausible causal SNP variants at these six loci mapped to *CADM2* on chromosome 3, *BAG6* on chromosome 6, *PHF2* and *FAM120AOS* on chromosome 9, *NCAM1* on chromosome 11, and *CKAP2, TPTE2P3*, and *DOCK9* on chromosome 13. Four of the six genes at the IBS loci were previously implicated in mood or anxiety disorders, including dedicator of cytokinesis 9 *(DOCK9)*; neural cell adhesion molecule 1 *(NCAM1)*, associated with neuroticism, anxiety, mood disorders, and anorexia nervosa; cell adhesion molecule 2 *(CADM2)*, associated with neuroticism, anxiety, and cannabis use; and PHD finger protein 2 *(PHF2)*. The *PHF2*-related protein FAM120AOS is associated with neuroticism, depression, and autism. *DOCK9* and *PHF2* also play a role in normal brain development. The role of *BAG6* and *CKAP2* in IBS is unclear and warrants further research. Using gene-expression co-localization analysis, the authors found that the variants regulate gene expression in a number of tissues, including the brain. *PHF2, NCAM1*, and *DOCK9* are also expressed in the gut. Although these genes are associated with IBS function in the enteric and central nervous systems, the authors emphasize that there are no gastrointestinal-specific genes identified in IBS patients nor genes that overlap with other intestinal disorders. Of the associated symptoms reported by participants, anxiety, neuroticism, depression, and schizophrenia showed the strongest genomewide overlap with IBS. The genetic correlation between IBS and anxiety was the strongest and independent of the other, reflecting a shared etiology as opposed to separate conditions. Statistical analysis showed a modest but low (less than six percent) SNP heritability for IBS. In contrast, the SNP heritability reported for Crohn's disease, ulcerative colitis, and anxiety was higher (41 percent, 23 percent, and 26 percent, respectively). These results suggest that factors other than SNP heritability that are shared within families may play a more prominent role in the

development of IBS.

Eijsbouts C, Zheng, T, Kennedy NA, et al. Genome-wide analysis of 53,400 people with irritable bowel syndrome highlights shared genetic pathways with mood and anxiety disorders. *Nat Gen*. 2021;53:1543–1552.

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Molecular mechanisms of circRNA C190 in modulating EGFR/ERK pathway in NSCLC

Circular RNAs are a recently identified form of noncoding RNAs that have increasingly generated scientific interest due, in part, to their role in regulating genes. They derive from a transcribed gene that undergoes noncanonical RNA splicing that results in the formation of a closed-loop RNA transcript. One of the most studied functions of circular RNA (circRNA) is its ability to act as a molecular sponge, or absorbent, to sequester and inhibit the function of noncoding microRNAs (miRNAs) that, in themselves, inhibit gene expression. Therefore, circRNAs promote protein biosynthesis by preventing miRNA-mediated gene silencing. In this era of targeted cancer therapy, one of the biggest challenges is developing gene mutations that confer tumor resistance to targeted therapy. For example, acquired resistance mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) in patients with nonsmall cell lung cancer (NSCLC) treated with first- or second-generation EGFR tyrosine kinase inhibitors result in tumor progression. Third- and fourth-generation EGFR tyrosine kinase inhibitors have been developed to respond to the acquired resistance. However, developing a better understanding of the molecular underpinnings regulating tumorigenesis, including the role of noncoding RNAs, may lead to the development of alternative therapeutic targets. The authors previously demonstrated that circRNA C190 can be detected in the peripheral blood samples of patients who have lung cancer, with elevated levels associated with tumor size, metastasis, and poorer prognosis. In this study, they established a baseline elevation of circRNA C190 levels in NSCLC tissue and lung cancer cell lines. The authors found that circRNA C190 levels increased through the EGFR/ERK/MAP kinase pathway in response to EGFR stimulation. In vitro overexpression of circRNA C190 resulted in a positive feedback loop of ERK activation with cell proliferation and migration, independent of EGFR activation. In vivo studies demonstrated larger tumor sizes in a xenograft animal model of lung adenocarcinoma with A549 cells overexpressing circRNA C190 as compared to control animals without circRNA C190 overexpression. In contrast, the specific reduction of circRNA C190 by CRISPR/Cas13a-mediated technology in lung cancer cell lines resulted in circRNA C190 degradation, reduced MAPK activation, prolonged cell survival, and smaller tumor sizes in xenografts from animal models as compared to controls. Transcriptome analysis revealed a large number of differentially expressed genes in response to circRNA C190 overexpression, with notable enrichment of such cyclin-dependent kinases (CDK) as CDK1, CDK2, and CKD4, all of which regulate phase transitions of the cell cycle. The authors verified the increased protein expression of the cyclin-dependent kinases CDK1, CDK4, and CDK6 and hyperphosphorylation of their downstream target—retinoblastoma (Rb) protein—in response to circRNA C190 overexpression. To evaluate how circRNA C190 overexpression resulted in elevated CDK protein levels, they performed computational searches to identify an miRNA likely associated with circRNA C190 and CDKs. MiR-142-5p was identified as the most likely miRNA candidate. Using in vitro assays, the authors demonstrated the direct interaction of miR-142-5p with circRNA C190. Functional studies showed reversal of miR-142-5p suppression of CDK6 expression by circRNA 190. These data confirm the antagonistic roles of circRNA 190 and miR-142-5p. Patient tumor samples demonstrated higher miR-142-5p expression levels in early stage lung cancer, whereas higher circRNA C190 and CDK expression levels were seen in later stages of the disease, in agreement with dysregulation and tumor progression. In summary, the authors provided a well-defined molecular mechanism of tumorigenesis modulated by noncoding RNAs in the EGFR/ERK pathway that may allow novel therapeutic targeting.

Adekunle IA, Chien C-S, Yang Y-P, et al. Oncogenic circRNA C190 promotes non-small cell lung cancer via modulation of the EGFR/ERK pathway. *Cancer Res*. 2021. doi:10.1158/0008-5472.CAN-21-1473

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