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Krüppel-like factor 12 promotes colorectal cancer via early growth response protein 1

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Results of preclinical studies by MUSC investigators reported in the July 2016 issue of *PLOS One*, demonstrate for the first time that the transcription factor KLF12 promotes CRC cell growth, in part, by activating EGR1. Furthermore, data demonstrate that KLF12 and EGR1 levels synergistically correlate with poor CRC prognoses.

CRC is the third most common and third deadliest cancer in the US. Like most cancers, CRC development is spurred by a series of genetic mutations and epigenetic changes that alter gene expression. In turn, this altered gene expression initiates tumors and supports their progression. Thus, transcription factors that regulate gene expression and signaling pathways during carcinogenesis have long been studied as potential therapeutic targets.

Dr. Raymond DuBois, Dean of the MUSC College of Medicine, Professor of Biochemistry and Molecular Biology, and senior author on the article is focused on understanding the role of inflammation in cancer. "We've been studying the connections between inflammation and cancer in my lab for some time now and have determined that some inflammatory mediators stimulate the progression of cancer," DuBois said. "We found that KLF12 was increased dramatically in the presence of inflammation in certain cancers, so we were trying to determine the specific molecular mechanisms responsible for these effects."

Other researchers who were studying kidney development previously identified transcription factor KLF12 as a transcriptional repressor of

the AP-2α gene. It was then discovered that AP-2α expression is also reduced in advanced CRC tumor tissue compared to matched normal tissue and that loss of AP-2α promoted CRC invasion. This connection illuminated a potential link between KLF12 and CRC. In vitro studies show that KLF12 promotes gastric cancer (GC) cell proliferation and invasion, and that KLF12 levels are elevated in about 40% of poorly differentiated GCs and correlate with tumor size. Furthermore, recent genome-wide analyses find high KLF12 levels in approximately 40% of esophageal adenocarcinomas and in 45% of salivary tumors. Until now, however, the role of KLF12 in CRC remained unclear.

The MUSC research team designed a series of in vitro and in vivo experiments to clarify the role of KLF12 in CRC. The first set of studies examined KLF12 expression in 7 human CRC cell lines. They found not only that KLF12 was expressed in 6 of the 7 cell lines, but also that its over-expression led to increased cell numbers and KLF12 knockdown led to reduced cell numbers. In addition, they also found that over-expression of KLF12 led to the formation of larger cecal tumors while KLF12 knockdown led to formation of smaller cecal tumors, compared to controls. Thus, this set of experiments indicates that KLF12 promotes CRC growth by enhancing cancer cell proliferation and/or survival.

The next set of experiments focused on clarifying which KLF12 target genes may be involved in regulating CRC growth. Using microarray assays, the researchers found that KLF12 over-expression altered multiple genes including EGR1. It has been previously reported that KLF12 regulates expression of some target genes by binding to the CACCC motif. They found that the EGR1 promoter contains two possible KLF12 DNA-binding motifs located at -1488bp (motif 1) and -808bp (motif 2) relative to the transcription start site. Using ChIP assay, the MUSC researcher team found that KLF12 does, indeed, bind strongly to the EGR1 promoter motif 2 but not to motif 1. In vitro experiments demonstrated that, at both the mRNA and protein levels,

CRC cells with undetectable levels of KLF12 expressed the lowest levels of EGR1 compared to cells expressing high levels of KLF12. In vivo studies using mice implanted with CRC tumor cells that over-expressed KLF12 showed that EGR1 expression was up-regulated compared to mice implanted with control cells. Furthermore, staining of human CRC tissue specimens produced the same pattern. Taken together, these results indicate that KLF12 directly activates EGR1 in CRC.

The third set of experiments looked at whether EGR1 mediated the effects of KLF12 on tumor cell growth. Results showed that EGR1 knockdown reduced KLF12-induced tumor cell growth, whereas EGR1 over-expression promoted CRC cell growth in vitro as well as tumor growth in the mouse model. The results of this set of studies, thus, indicate that KLF12 enhances CRC cell growth by activating EGR1.

The final set of experiments evaluated whether KLF12 and EGR1 levels correlate with CRC patients' prognoses. Using gene expression data from publicly available microarray databases (Moffitt [n = 177]; Vanderbilt Medical Center [n = 55]), CRC patients were stratified by level of KLF12 and/or EGR1 expression. These data showed that patients with high levels of either KLF12 or EGR1 had worse outcomes compared to those with low levels of these genes, and that those with high levels of both KLF12 and EGR1 had the lowest survival rates.

This is the first study to clarify the role of KLF12 in CRC tumor growth and progression which appears to occur, at least in part, through EGR1 activation. The finding that synergistic contributions of KLF12 and EGR1 produce the worst outcomes among CRC patients illuminates their potential in developing novel therapies. More studies are needed to further clarify the role of KLF12 in CRC progression and its potential as a novel prognostic marker and therapeutic target.