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Student ID : 1931D01

Name : Yui Masumoto

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Dissertation Abstract

Cancer statistics from the National Cancer Center in Japan indicated that colorectal cancer ranked first among organ-specific cancer cases in 2019, demonstrating high incidence rates in men and women. These rates are anticipated to continue increasing. The multistep accumulation of abnormalities in various oncogenes and tumor suppressor genes caused colorectal cancer, making the identification of the causative genes of colorectal cancer crucial.

We conducted a comprehensive analysis of the genes expressed in mouse intestinal tumors using $Apc^{Min/+}$ mice, a human colorectal cancer model, in our laboratory and revealed a highly expressed *Trefoil factor (TFF) 2. TFF2* is normally highly expressed in gastric tissue in human. It is considered a secretory protein, a small peptide with a molecular weight of 12 kDa, secreted by mucus-producing cells in the gastrointestinal tract and other tissues. *TFF2* has conserved functional domains in humans, mice, rats, and cattle. Recent experiments using mice have indicated that the TFF2 protein in the pancreas during embryonic development plays a crucial role in organogenesis. Furthermore, decreased *TFF2* expression has been reported in gastric cancers, whereas high *TFF2* expression has been observed in many tumors, including pancreatic and colorectal cancers. Conflicting opinions indicated its cancer-promoting or suppressive role and the details remain unclear.

Herein, I conducted experiments divided into four chapters to determine the function of *TFF2*. In Chapter 1 focuses on the high *TFF2* expression in intestinal tumors *in vivo* and its absence in cultured colorectal cancer cells *in vitro*, thereby prompting a reconsideration of the cell culture conditions. The result of mimicking conditions of the tumor microenvironment, such as temperature fluctuations and acidic pH, indicated that *TFF2* expression is significantly induced by acidity, and this expression was confirmed to increase acidic pH-dependently. I revealed that the high *TFF2* expression, typically observed in normal gastric tissue, is induced not only in normal tissues but also in tumor cells under acidic conditions. Chapter 2 comprehensively analyzes the expression patterns of other gene groups under acidic conditions. Additionally, I conducted analyses to confirmed an increase in the expression of gene groups related to plasma membrane, glycoproteins, and cell surface under acidic conditions. These results indicate that cells increase the expression of membrane components in response to their surrounding environment, thereby facilitating processes such as repair. The TFF2 protein, present in the culture supernatant, significantly increased the expression of genes that encode extracellular matrix fibronectin III domain and actin-binding proteins in surrounding cells. These results indicate that the secreted TFF2 protein plays a role in increasing extracellular matrix components such as

fibronectin, thereby facilitating scaffold formation during organogenesis. Chapter 3 examines the changes in the expression of these genes when *TFF2* was suppressed using siRNA, considering the involvement of the TFF2 protein in upregulating fibronectin-related gene expression. The comparison before and after *TFF2* suppression revealed significant *FNDC3B* and *FNDC5* inhibition, which encode fibronectin type III domain proteins. FNDC3B protein has been involved in proliferation and invasion through the PI3K/mTOR signaling pathway in intestinal tumors. Therefore, targeting *TFF2* to suppress its expression will inhibit invasion effects. Chapter 4 conducted metabolomics analysis to determine the effect of the TFF2 protein present in the culture supernatant. Pathway analysis revealed significantly elevated metabolites of the taurine-hypotaurine and glutathione pathways. Taurine has improved mitochondrial function and suppressed apoptosis through its antioxidant properties, whereas glutathione popularly maintains cellular homeostasis. These results indicate that *TFF2*, whose expression increase under acidic conditions, promotes the upregulation of extracellular matrix fibronectin expression in surrounding cells. Furthermore, *TFF2* may facilitate metabolite production that contribute to stress resistance and cellular homeostasis in acidic environments.

TFF2, which plays a crucial role in pancreatic organogenesis and gastric mucosal protection, has been subjected to numerous conflicting reports regarding its role as either promoting or inhibiting tumor growth. Over the years, its role in tumors has remained unclear. This study revealed that *TFF2* is induced in tumors by the acidic tumor microenvironment through the Warburg effect, thereby playing a critical role in cell survival. The high *TFF2* expression in tumor tissues was considered a tumor progression indicator. Additionally, it indicated the potential for effectively targeting tumor cell survival inhibition as a therapeutic approach for cancers.