Hypotheses

State of the research

Role of atrial natriuretic peptide receptor in inhibition of laterally spreading tumors via Wnt/β-catenin signaling

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Abstract

Colorectal cancer (CRC) is the third most prevalent malignancy worldwide. Laterally spreading tumors (LSTs), as special manifestations of digestive tract tumors, are often misdiagnosed or undiagnosed due to their unique morphological and pathological features. LST has no protruding lesions and progresses rapidly, and prognoses are consequently poor. LST progression to CRC is complicated. Clinical data indicate that the heart is rarely the site of primary tumorigenesis, and a class of atrial natriuretic peptides (ANPs) secreted by heart tissue play an important role in this phenomenon, which is closely related to the Wnt/β-catenin signaling pathway. However, previous studies focused solely on correlations between the Wnt/β-catenin signaling pathway, downstream gene expression and LST. Thus, correlational studies of ANP/ANP receptor, LST and CRC may be of great help in understanding the occurrence, development and treatment of LST, as well as in establishing specific and sensitive methods for detecting LST.

Key words: colorectal cancer, laterally spreading tumor, ANP/ANPR, Wnt/β-catenin signaling pathway, gene expression.

Introduction

Colorectal cancer (CRC) is a common malignant tumor of the digestive system, and its incidence in China and internationally is increasing. CRC has the third highest incidence of all malignant tumors, and developed countries have higher incidence than developing countries [1]. Large intestine adenomatous polyposis is one of the main precancerous lesions of the large intestine, and originates in the large intestinal mucosa. These lesions grow in the cavity and can be found and removed in early stages during colonoscopy. Thus, removal of adenomatous polyposis represents the gold standard for preventing large intestine cancer [2].

In 1993, Japanese scholars identified a class of lesions characterized by lateral superficial growth along the surface of the large intestine. The surface had a particle-like feature and it was classified as a cluster-like lesion of the large intestine [3]. The lesion is also known as a superficial extension type tumor, a rhabdoid tumor or a petal-like lesion [4]. With additional study, the non-particle type of the lesion was documented and it was formally designated [5] as a large intestine lateral spreading tu-

mor (LST). LSTs are defined as shallow lesions with a diameter of more than 10 mm and nonvertical growth, with both particle and non-particle-type surface morphology [6]. Using morphological definitions, LST is classified as both a benign tumor and a malignant tumor, and the pathological classification is mainly mucosal cancer and adenoma. LSTs may occur in parts of the digestive tract other than the large intestine [7]. Under the endoscope, LSTs can be divided into granular and non-granular forms according to surface morphology; the former can be divided into a uniform particle type and a nodular mixed type, and the latter can be divided into a false depression type and a flat bump type [8]. The uniform particle type can be observed under the endoscope: the particles are of basically equal size and shape, and the surface particles have diameters of less than 3 mm. In the mixed type, the surface particles have nodes and sizes that differ. The flat raised type manifests as a flat-like lesion under the endoscope, with a petal-shaped pseudo-foot that can be extended into the periphery. The false depression type is less common. A flat lesion with a mild depression in the center has also been observed.

According to the literature, benign LST lesions can develop into advanced CRC within 3 years, and the incidence rate of benign LSTs is significant in China. Lower detection rates in the past may be related to missed diagnoses or misdiagnoses and insufficient recognition of improperly applied methods [9]. The incidence of LST is high in individuals between 50 and 70 years old, and increases with advancing age [10]. Development of granular LSTs in the right colon and rectum is apparently more likely than in other locations, while nongranular LSTs are not significantly associated with any site. The missed diagnosis rate of right colon lesions by colonoscopy is higher than that in the left colon, which is one potential reason for LSTs being easily missed [11]. The likelihood of patients with LSTs developing CRC was 8.4% to 52.5% [12], according to a large-scale controlled study. A study of 428 patients with LSTs and submucosal carcinoma found that the proportion of false depressions was the highest, and when the diameter of the lesion was larger than 20 mm, the probability of it developing into submucosal carcinoma of the false-depressed type LST increased significantly, to 46% [13]. The malignant potential of various types of LSTs differ. It has been reported that the malignant potential of flat protruding lesions is higher than that of granular lesions, but the size of the lesions is not an influencing factor. There was no significant difference between tumorigenesis of granular lesions with larger diameters and polyposis lesions with smaller diameters [14]. A large group of 1920 patients was examined by enteroscopy in China and the results showed that the incidence of LST lesions was 0.8%; 1.5% of LST patients developed submucous carcinoma, suggesting that LST has a high incidence in the Chinese population [15]. Taken together, the data suggest that the detection rate of LSTs is between 8% and 12%, based on advanced diagnostic equipment and rich clinical experience. In clinical practice, the detection rate of this disease is much lower than this value, so early specific diagnosis of LST is particularly important and necessary.

Molecular biological characteristics of LST

The progression of LSTs to CRC requires a series of processes, including adenoma formation, which are regulated by a variety of proto-oncogenes, tumor suppressors, apoptosis-related genes and other factors. It has been reported that at least five to six genetic changes occur during the evolution of CRC [16]. Therefore, the study of molecular biological characteristics is of great significance to understand the process and mechanisms of LST transformation.

Proto-oncogenes involved in LST development

As an important member of the Myc gene family, c-Myc is not only a differentially expressed gene in cancer but is also involved in a number of chromosomal translocations. During cellular differentiation, c-Myc mainly plays a negative regulatory role. Abnormal expression can lead to malignant transformation, as well as unlimited proliferation, resulting in tumor formation. Some studies have shown that c-Myc plays a key role in the occurrence and development of a variety of tumors. In many tumors, such as colon cancer, breast cancer, and malignant lymphoma, c-Myc gene amplification, rearrangement or overexpression has been documented [17]. C-Myc can induce cellular transformation and tumorigenesis *in vitro*. However, its amplification in the human body is coordinated with changes in other genes in the early stages of CRC, which is more common in highly malignant tumors with poor prognoses. It has been reported that the abundance of c-Myc mRNA in LSTs is 3- to 24-fold higher than that in normal colorectal mucosa [18]. One way c-Myc regulates the cell cycle, which determines its mRNA expression level, is through the p21 protein (cyclin-dependent inhibitor-1).

The Ras protein is highly conserved. Upon interaction with guanine-containing nucleotides, Ras can take on an active form in complex with GTP, but can also be expressed as an inactive form in complex with GDP. Conversion between the two forms is an important hub for the Ras protein to participate in regulation of cell function and signal transduction [19]. The conversion process is reg-

ulated by a variety of protein factors as well as by Ras endogenous GTPase activity. Some scholars have found that mutation of residues 12, 13 and 61 of p21ras was associated with the occurrence of LSTs, and that p21ras-GTP cannot be hydrolyzed to GDP [20] owing to a lack of GTPase enzyme activation. It was also reported that the ras gene mutation rate was 7% in early adenomas with diameters of < 1 cm and 57% in medium-term adenomas with a diameter of 1 cm. Ras gene mutations are likely initiation events of LST malignant changes. However, LSTs caused by ras gene mutations are not significantly correlated with the patient's family and gender [21].

Tumor suppressor genes involved in suppressing LST

The main biological functions of the p53 gene are to repair cellular injury and to monitor the integrity of genomic DNA. When DNA is damaged by drugs or radiation, p53 can stop the process of cell division in G1/S phase to help cells to repair the damage prior to division. For irreparable DNA damage, p53 can trigger programmed cell death by initiating apoptosis, avoiding the production of mutant cells that may induce carcinogenesis [22]. The mutation rates of the p53 gene in colorectal adenoma and LSTs are about 20% and 18.2%, respectively, indicating that p53 gene mutations may occur during the middle and late stages of carcinogenesis. The large number of p53 mutations reduces the proportion of wild type p53, and its function in monitoring the integrity of genomic DNA is also weakened, providing conditions for tumorigenesis. Expression of p53 in LSTs is upregulated, suggesting that p53 plays a role in the process of benign LST carcinogenesis [23]. Other scholars have found that p53 expression was correlated with LST lymph node metastasis [24].

At present, it is believed that APC, as the "doorman" gene of colorectal epithelial tissue, plays an important role in maintaining the mucous epithelial cell number. APC is also the key rate-limiting gene for tumor transformation, and can regulate levels of b-streptavidin through two b-linked peptide binding regions. Thus, APC is an important gene in the pathogenesis of colorectal tumors [25]. The polymerase chain reaction single-strand conformation polymorphism technique was applied to assess the frequency of APC gene mutations in the normal colorectal mucosa, LST, colorectal carcinoma and colorectal adenoma. Almost no APC gene mutations were detected in normal colorectal mucosa, but the APC mutation rates were 25%, 30% and 27.8% in LST, colorectal adenoma and colorectal carcinoma, respectively. The mutation rates of the APC gene in LSTs reported in the literature were 15.5% to 42.4%, suggesting that APC gene mutations played a key role in the occurrence and development of LSTs, and may represent early molecular drivers in the process of carcinogenesis [26].

Apoptosis-related genes involved in LST

The Bcl-2 gene is of significant interest owing to its role in regulating apoptosis. Bcl-2 is a cytoplasmic membrane protein with a molecular weight of 26,000 Daltons, is encoded at 18q21, and is widely expressed during the activation and development of normal cells. Bcl-2 is rarely expressed in mature cells or apoptotic cells [27]. It has been confirmed that Bcl-2 can inhibit apoptosis by regulating mitochondrial activity. In eukaryotic cells, Bcl-2 gene expression products and the homologous protein Bcl-XL can play an inhibitory role in apoptosis induced by many factors. When expression of Bcl-2 is low or deleted, the mortality of damaged cells will increase significantly. Expression of the Bcl-2 gene in colorectal adenoma was similar to that in LST, which may be related to the normal loss of Bcl-2 expression during colorectal epithelial differentiation. The persistent high expression of Bcl-2 in surface epithelial cells is closely related to the occurrence of LSTs [28].

The survivin gene is not expressed inhuman normal tissues (except in the thymus) but is expressed in most tumor tissues and is a unique mammalian apoptosis-inhibiting gene. Survivin gene expression can be detected in various transformed cell lines *in vitro*, and expression of survivin in human tumor tissues, such as lung cancer, breast cancer and colon cancer, can be observed. In LST tissue, high survivin expression is detectable [29]. This suggests that during the early CRC events, expression of survivin, which is activated in the LST stage, has an inhibitory effect on apoptosis. Thus, survival of tumor cells is prolonged and the development of CRC is promoted. Therefore, survivin has potential value as a specific diagnostic index for LST, and may represent a new target for the treatment of CRC [30].

At present, at least 11 human caspase family members have been identified. As apoptosis executioner genes, the activation and regulation of caspase family genes directly affect the occurrence and biochemical characteristics of apoptosis. The gene products of this family are produced in the form of inactivated precursors. When the caspase protease is activated to form tetramers, enzymatic hydrolysis can occur [31]. It has been suggested that caspase proteases can auto-activate and activate one another, and that Ca^{2+} can activate them directly or indirectly. However, the specific processes and regulation of their activation into tetramers need to be further studied [32]. Caspase-9 is widely expressed in the cytoplasm of the normal colorectal mucosa, and its expression in LST is significantly higher than that in adenocarcinoma. This

may be related to a decrease of caspase-9 expression and inhibition of apoptosis, resulting in tumor growth and deterioration of LST [33].

Heart biofunctions and atrial natriuretic peptides (ANPs)

Of human tissues and organs, few primary tumors occur in the heart and its adjacent arteriovenous tissues. Malignant primary tumors and tumors with recurrent metastases and implantation are rarely reported, potentially because of the following factors. Myocardial tissue, conduction tissue and supporting tissue are mostly composed of terminally differentiated cells; the morphologies and functions of these cells are very stable and are not easy to transform. These cells and tissues are not easily invaded by foreign cells. To promote blood circulation, the heart is a high energy consumption organ, and the active absorption of energy makes it difficult for malignant tumors to grow. Because of the high pressure of blood flow in atrial ventricles and the aorta and the rapid flow rate, the probability of invasion and implantation of tumor cells is very low [34, 35].

The above functions and structures macroscopically explain the very low incidence of primary and metastatic recurrence of the heart. However, apart from being an energy-supplying organ, the heart is demonstrated to have endocrine function, playing a vital role in metabolism, and influencing metabolic disease [36–38]. For instance, a family of small molecular weight peptides secreted by cardiac tissue also plays an important role in the very low incidence of cardiac malignancies.

Natriuretic peptides (NPs) are a family of cardiac hormones that includes atrial, brain and C-type NPs (ANPs, BNPs, and CNPs, respectively). ANPs and BNPs are mainly produced in the atria and ventricles of the heart and play an important role in maintenance of cardiovascular stability [39]. ANP is stored as a pro-peptide in the dense particles of cardiac myocytes. Atrial lengthening caused by hypertension leads to release of ANP into the bloodstream [40]. ANP is synthesized as an inactive precursor and is hydrolyzed by a membrane-associated protease, Corin, to convert it into the mature active peptide [41]. The biological effect of NPs occurs mainly through activation of the ornithine-acid cyclase A and the B-receptor through an intracellular messenger cGMP-mediated series of biochemical reactions.

In recent years, it was discovered that ANP is not only expressed in the heart, but is also produced in the colon, kidney, articular cartilage, ovary and other organs. ANP is involved in anti-inflammatory immune responses and in regulating metabolism [42]. The biological functions of ANPs include anticancer effects. ANPs can slow progression of prostate, breast, pancreatic and colorectal adenomas. At present, it is believed that the potential anticancer mechanisms of ANPs include inhibition of DNA synthesis through intracellular messenger DNA [43], and they may have a variety of other anticancer effects. The molecular mechanisms of antitumor and antiproliferative activity may be related to effects on the expression of biomolecules including Ras-MEK1/2, ERK1/2, the Wnt pathway, vascular endothelial growth factor, and β-catenin [44]. The ANP receptor (ANPR) is a guanylate cyclase receptor on the cell surface and is expressed in different organs and tumor tissues. ANP binds to ANPR and exerts biological effects.

Combining the molecular biological characteristics of LSTs and the mechanisms of the antitumor effect of the ANP/ANPR system, we found that both LST pathogenesis and the therapeutic mechanism of ANPs were significantly dependent on changes in the Wnt/β-catenin signaling pathway as well as abnormal expression of downstream effectors. Therefore, we propose in this study to establish a correlation between ANP/ANPR expression level and expression of molecules of the Wnt/β-catenin signaling pathway as well as its downstream effectors. This will achieve the following two objectives: (i) to establish a specific and sensitive ANP/ANPR detection method for LST, and (ii) to explore the evidence supporting ANP as a target for biological intervention in LST, which is needed to design new biotherapies for LST.

In conclusion, by establishing correlations between ANP/ANPR expression and expression of Wnt/β-catenin signaling pathway members and their downstream effectors, we hope to develop a specific and sensitive method for detection of LST mediated by ANP/ANPR. Based on the inhibitory function of ANP/ANPR on tumors, we will explore new molecular evidence supporting the biological role of ANP/ANPR in LSTs, and provide new ideas and methods for biotherapy of LSTs.

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Conflict of interest

The authors declare no conflict of interest.

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