Review

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Research Advances in the Role of Keratins in Gastrointestinal Cancer

Siwen Ouyang[1,2], Weiming Kang[2*]

[1]Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China
[2]Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100010, China

ABSTRACT

The incidence and mortality rates of gastrointestinal (GI) cancer remain high. Despite constant improvements in diagnostic and therapeutic techniques, the early diagnosis, mid- and late-stage treatment, drug tolerance, and cancer recurrence and metastasis in GI cancer remain challenging. In this review article we summarize the recent research advance in the roles of keratins in GI cancer, with the hope that they will become efficient biomarkers for the prediction, diagnosis, or treatment of these malignancies.

Key words: Keratin, gastrointestinal cancer, diagnostic significance, mechanism

INTRODUCTION

Keratins, or cytokeratins, are components of cytoskeletal protein intermediate filaments. The keratin family consists of more than 50 members and plays an important role in maintaining the mechanical stability and integrity of epithelial cells[1]. Keratins are usually expressed in epithelial cells and tumor tissues of epithelial origin[2]. The relationship between keratins and malignant tumors, the family characteristics of keratins, and the abnormal expressions of proteins encoded by keratin family genes and gene mutations have long been hot research spots in biology and oncology. Keratins often lead to the occurrence and development of a variety of diseases, with poorly defined mechanisms. In this review article, we summarize the recent research advance on keratins, with the hope that they will become efficient biomarkers for the prediction, diagnosis, or treatment of gastrointestinal (GI) cancer.

MAJOR GASTROINTESTINAL CANCERS

Gastric cancer is one of the most common malignant tumors globally. In 2018, there were about 1.03 million new cases of gastric cancer worldwide, ranking fifth among all malignant tumors[3]. The incidence and mortality of gastric cancer ranked third in China[4]. About 80% of patients are already in the advanced stage at diagnosis, and the overall 5-year survival rate is below 50%[5]. Colorectal cancer is another common malignant tumor of the digestive system, ranking third in both the global incidence and cancer-related deaths. The five-year survival rate for patients with distant metastases is only 11.7%[6,7]. Although adjuvant treatments such as chemical drugs and molecular targeted drugs have brought many survival benefits in recent years, the treatment tolerance and postoperative recurrence and metastasis remain problematic for GI cancer patients.

OVERVIEW OF KERATINS

Keratin family

The cytoskeleton is composed of intermediate filaments (IF), microtubules, and microfilaments. The diameter of IF is between the microtubules and microfilaments.

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*Corresponding author E-mail: pumchkangweiming@126.com.

IF proteins are specifically expressed in tissues and cells. They can be divided into six categories based on the difference in protein structure and amino acid sequence. With more than 50 members, keratins make up the largest subgroup of IF and are divided into type I and type II. The protein structures of keratins are similar to other IF proteins, with an α-helical domain in the middle and non-helical head and tail domains at both ends. Most of the regulatory sequences, such as phosphorylation, ubiquitination, and glycosylation sites, exist in the head and tail domains, which are also regions of structural heterogeneity between different keratin molecules[8]. Most keratins are encoded by chromosome 12 or chromosome 17 and can be divided into type I and type II according to different isoelectric points. The isoelectric point (PI) of type I (acidic) keratins range 4.9 - 5.4, while the PI of type II (neutral) keratins range 6.5 - 8.5[9]. A keratin is composed of at least one type I and one type II keratin molecules, which are combined into heterodimers by non-covalent bonds.

Physiological functions of keratins

Keratins are vital for epithelial cells. They maintain the structural integrity of epithelial cells and tissues, establish cell polarity, and protect cells and tissues from fluid dynamic pressure changes[10]. In addition, keratins are also involved in determining cell size, regulating signal transduction, regulating translation and expression, and affecting cell proliferation, cell-specific organelle transport, malignant transformation, and various stress responses[11]. Cells can respond to different stress responses by changing the expression levels of keratins. For example, under stress conditions, the expression level of epithelial keratin KRT8/KRT18 increases twice, which enhances the protective effect of liver cells[12]. During skin wound healing, differentiated epidermal keratinocytes rapidly inhibit the expressions of KRT1 and KRT10 and promote the expressions of KRT6, KRT16, and KRT17[11]. After phosphorylation, KRT8/KRT18 can bind to 14-3-3 protein to participate in cell signal transduction. Although the mechanism that regulates cell proliferation and cell size is unclear, the mTOR pathway and 14-3-3 protein have shown important roles[13]. Changes in the expressions of certain keratins have notable impacts on cell size and proliferation. For example, KRT17 can regulate cell size and inhibit the expression of KRT17 in keratinocytes, resulting in a reduction in cell volume and a reduction in total protein synthesis[14]. Keratins can regulate the location of AP-3 complexes and the interaction between AP-3 vesicles and motor proteins, thereby affecting the transportation and positioning of melanosomes. The direct interactions between keratins and 14-3-3 protein can also affect the transport of melanosomes[11]. Keratins are also involved in the tolerance of cells to stress and apoptosis. For example, mutation of KRT8 gene causes its protein overexpression, which will increase the individual's susceptibility to stress-induced liver injury and apoptosis[15].

RELATIONSHIP BETWEEN KERATINS AND GASTRIC CANCER

Keratins affect the progression of gastric cancer and the underlying mechanisms

Keratins can be expressed either in normal epithelial cells and skin hairs and specifically in tumor tissues. In gastric cancer cells, Gal-9 protein can increase the expression of KRT18 protein, leading to apoptosis and inhibiting the proliferation of gastric cancer cells[16]. The effects of keratins on gastric cancer are also associated with immune regulation. The absence of KRT76 can up-regulate the expression of pro-inflammatory cytokines and regulate the accumulation of Treg cells (Tregs) in the tumor microenvironment, thus enhancing the cancer sensitivity of gastric cells to carcinogens[17].

The abnormal expressions of keratins in gastric cancer are often related to the AKT/mTOR pathway, which is often activated in aggressive tumors. Keratin-mediated AKT may play an important role in the occurrence of gastric cancer. AKT subunits can regulate the expression of intermediate filaments in cancer cells. For example, overexpression of AKT1 can up-regulate the protein expression levels of KRT8 and KRT18, and AKT2 can up-regulate KRT18[18]. Studies have reported that keratins can act on the mTOR pathway. In keratin gene knockout mice, due to the abnormal location of the glucose transporter, the activation of adenosine phosphate-activated kinase, which in turn inhibits the kinase downstream of the mTOR pathway, eventually leads to severe developmental delay and even embryonic lethality[19]. Protein post-translational modification also plays a vital role, and it has been found that sphingosylphosphocholine

(SPC) induces protein phosphorylation of KRT8, which enhances the metastatic ability of gastric cancer cells[20].

Diagnostic significance of keratins in gastric cancer

As immunohistochemical markers, keratins have long been used in the pathological diagnosis of gastric cancer[21]. KRT17 can activate the AKT/mTOR pathway in gastric cancer cells, leading to the proliferation and metastasis of gastric cancer cells, marking the significance of KRT17 as a tumor biomarker and molecular therapy target[22]. Many studies have investigated the up-regulation of keratin transcription level in gastric cancer. For example, the transcription level of KRT7 was significantly up-regulated in gastric cancer cell lines infected with *Helicobacter pylori*[23]. In gastric cancer cells, the transcription levels of genes such as KRT6A, KRT13, and KRT17 were up-regulated[24]. KRT20 expression was up-regulated in peripheral blood and lymph nodes harvested from 85 gastric cancer patients, and it was associated with poor clinical prognosis[25]. KRT7 and KRT20 were usually positive in gastric cancer[26]. KRT40 was related to the clinical prognosis of gastric cancer patients[27].

RELATIONSHIPS BETWEEN KERATINS AND COLORECTAL CANCER

Keratins affect the progression of colorectal cancer and the underlying mechanisms

The pathogenesis of colorectal cancer involves the gradual progression from normal intestinal epithelium to malignant tumors, with the accumulation of genetic abnormalities at each step, which involves the classical chromosomal instability pathway or the microsatellite instability pathway[28]. A study on the expressions of keratins in signet ring cell carcinoma (SRCC) of the gastrointestinal tract found that certain expressions of keratins were knocked out in colorectal SRCC, which were often expressed as $KRT7^-/KRT20^+$, $KRT7^-/KRT19^+$, and $KRT7^-/KRT20^-$[29]. Therefore, the abnormal expressions of keratins in intestinal cells may be an important mechanism in colorectal carcinogenesis.

Keratins are abundantly expressed in colorectal tissues, and the abnormal expressions and functional changes are closely related to the progression of colorectal cancer. Dephosphorylation of KRT8 is closely related to the progression of colorectal tumors[30]. Before colorectal cancer cells migrate and invade other tissues and organs, the expression of PRL-3 is increased and the phosphorylation of KRT8 is reduced or absent, indicating that dephosphorylation of KRT8 can increase the aggressiveness of tumors. Julia *et al.*[31] found that KRT8 bound to inflammasomes in the colonic epithelium *in vivo*, and the lack of KRT8 lead to the activation of inflammasomes and further activation of IL-22, JAK/STAT pathways, and epithelial defense mechanisms.

Keratins also have an effect on the growth of cancer stem cells. It was found that KRT23 promoted the stemness of colon cancer stem cells and increased the expression of CD133 and CD44, and it promoted the proliferation and metastasis of colon cancer cell lines through the p38 and ERK 1/2 pathways[32]. KRT19-positive cancer-initiating cells are radioresistant, while Lgr5-positive stem cells are radiosensitive, and therefore KRT19 can be used as a new target for the treatment of colorectal cancer[33].

Diagnostic significance of keratins in colorectal cancer

The abundant keratins expressed in intestinal epithelial cells mainly include KRT8, KRT18, KRT19 and KRT20[34]. Some keratins may have different expression profiles in the same tissue. For example, KRT8 is mainly expressed in the flat mucosa in the normal colon, and the expression gradually decreases from the top of the crypt axis to the base[35]. Imai *et al.*[36] found that the expression of KRT20 is closely related to the infiltration of poorly-differentiated adenocarcinoma, and it is an independent prognostic factor of poorly-differentiated colorectal adenocarcinoma. The keratin expression profiles discovered by Yamagishi *et al.*[37] were $KRT7^-/KRT20^+$ in high-moderately differentiated adenocarcinoma, mucinous adenocarcinoma, and signet ring cell carcinoma and $KRT7^-/KRT20^-$ in undifferentiated adenocarcinoma. However, the relevant findings were diverse for poorly-differentiated adenocarcinoma.The survival analysis showed that the non-expression or low expression of KRT20 in poorly differentiated adenocarcinoma is closely related to the prognosis of patients[37].

In an animal experiment, KRT8-deficient mice exhibited anti-apoptosis effect in the colon compared with wild-type mice[38]. The expressions of keratins

have important roles in human body, especially in the occurrence, development, and prognosis of colorectal cancer. Wang *et al.*[39] found that serum KRT18 fragment level was significantly higher in patients with rectal cancer than in the normal control group. Thus, serum KRT18 has high clinical diagnostic value and may be used as a marker for monitoring tumor response to treatment and evaluating patient prognosis.

Research on the expression of KRT19 in human fecal cells revealed that the average expression level of KRT19 in the stool of patients with colorectal cancer was significantly higher than that in normal people, and the combination of KRT19 expression in the stool and carcinoembryonic antigen (CA19-9) in the serum could predict the overall survival rate of elderly patients with colorectal cancer[40]. Moreover, the overexpression of KRT20 was closely related to the poor prognosis and decreased survival rate of colorectal cancer patients[41-42]. Since KRT20 was correlated with the clinical stage of colorectal cancer, it might be a marker for monitoring circulating colorectal tumor cells[41-42].

PROSPECTS

The past few decades have witnessed the constant research advances in keratins in terms of structures, classification, functions, and clinical applications. While the molecular and cellular characteristics of keratins are diverse, some keratins have been used to be prognostic factors and clinical biomarkers. However, the roles of keratins in signal transduction and the specific biological mechanisms and molecular behaviors need to be further explored, and whether keratins can be used as therapeutic targets remains controversial.

Currently, most of the keratin-related diseases are caused by genetic mutations. However, the more meaningful research is to explore the roles of keratins in tumorigenesis and as diagnostic tools and therapeutic targets. So far, most studies on keratins in gastrointestinal tumors are focusing on their prognostic values and the possibility of being molecular markers. The relationships of keratins with the occurrence and development of gastrointestinal tumors and the underlying molecular mechanisms still need further investigations. More effective biomarkers will help the early diagnosis and early treatment of gastrointestinal tumors and guide the prediction of prognosis. Eventually, research on keratins will provide a more clinically valuable basis for the diagnosis, treatment, and prognosis evaluation of gastrointestinal tumors.

Conflict of interests

The authors declare no conflict of interests.

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综述

角蛋白在胃肠道肿瘤中的作用和研究进展

欧阳思文[1, 2]，康维明[2*]

[1] 中国医学科学院北京协和医学院，北京 100730，中国
[2] 中国医学科学院北京协和医院基本外科，北京 100010，中国

摘要

胃肠道肿瘤的发病率和死亡率居高不下。尽管诊断和治疗技术在不断改进，胃肠道肿瘤的早期诊断、中期和晚期治疗、药物耐受性以及癌症复发和转移等问题仍构成巨大挑战。在本篇综述中，我们总结了角蛋白在胃肠道肿瘤中的作用和研究进展，希望它们能成为预测、诊断或治疗胃肠道肿瘤的有效生物标志物。

关键词：角蛋白；胃肠道肿瘤；诊断意义；机制

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通讯作者：康维明 pumchkangweiming@126.com