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Research Progress of HER-2 in Colorectal Cancer

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Abstract: Human epidermal growth factor receptor 2 (HER-2) is a transmembrane receptor tyrosine kinase that is overexpressed in various solid tumors and is closely related to tumor invasion, metastasis, and poor prognosis. In recent years, the expression of HER-2 in colorectal cancer and its clinical significance have gradually attracted attention. This article reviews the expression of HER-2 in colorectal cancer, the clinicopathological characteristics of HER-2 positive colorectal cancer, the detection methods of HER-2, and the drug treatment targeting HER-2, to provide references for clinical diagnosis and treatment and research.

Keywords: Human epidermal growth factor receptor 2; Colorectal cancer; Expression; Targeted therapy

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1. Introduction

Colorectal cancer is one of the malignant tumors with high incidence and mortality worldwide. Its pathogenesis has not yet been fully elucidated. Human epidermal growth factor receptor 2 (HER-2) is a transmembrane receptor tyrosine kinase encoded by an oncogene, playing an important regulatory role in cell proliferation, differentiation, and apoptosis. Recent studies have found that HER-2 is overexpressed in various solid tumors, with an expression rate of up to 20% to 30% in breast cancer, and is closely related to tumor invasion, metastasis, and poor prognosis. Currently, drugs targeting HER-2 have become the standard treatment for HER-2-positive breast cancer patients. In contrast, the research on HER-2 in colorectal cancer has been relatively lagging, but has gradually gained attention in recent years.

2. Expression of HER-2 in colorectal cancer

The HER-2 gene is located on the long arm of chromosome 17 (17q12-q21) and is encoded by 28 exons. The HER-2 protein is localized on the cell membrane and consists of three parts: the extracellular region, the

transmembrane region, and the intracellular region. When the ligand binds to the extracellular region, it can induce the dimerization of the HER-2 protein, thereby activating the tyrosine kinase activity in the intracellular region, initiating downstream signaling pathways, and thereby regulating the proliferation, differentiation, migration, and other functions of the cells [1].

The expression rate of HER-2 in colorectal cancer tissues varies and shows significant differences. He *et al.* conducted a meta-analysis on 123 colorectal cancer patients and found that the positive expression rate of HER-2 was significantly higher than that in normal tissues adjacent to the cancer ($P < 0.05$). The expression level of HER-2 is closely related to the clinical and pathological parameters of the patients. The combined detection of LGR5 and HER-2 has a high predictive value for the prognosis of patients [2]. Chen *et al.* analyzed 35 patients with benign colorectal lesions (benign lesion group) and 36 healthy individuals undergoing physical examinations (normal control group) for colorectal cancer. The serum HER-2 level was related to RAS gene mutations and peripheral nerve invasion in colorectal cancer patients ($P < 0.05$), while the CD44 level was not related to RAS gene mutations and peripheral nerve invasion ($P > 0.05$) [3]. The areas under the curve (AUC) for the individual and combined detection of HER-2, CD44, carcinoembryonic antigen (CEA), and carbohydrate antigen (CA19)-9 in the diagnosis of colorectal cancer were 0.779, 0.692, 0.620, 0.634, and 0.837 [4], respectively. It can be seen that the expression of HER-2 in colorectal cancer shows significant heterogeneity, and its positive rate is affected by factors such as region, stage, and tissue origin. Overall, it is approximately 5%.

3. HER-2 detection methods

Accurate assessment of HER-2 status in colorectal cancer is of great significance for guiding treatment decisions. Currently, the main methods for HER-2 detection include immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and real-time PCR, etc.

3.1. IHC

IHC (Immunohistochemistry) is the preferred method for detecting HER-2 expression. Its principle involves using specific antibodies to bind with the HER-2 protein, and through a color reaction, observing the expression of HER-2 on the tumor cell membrane under a microscope. Commonly used antibodies include HercepTest, 4B5, A0485, etc. Among them, HercepTest is a reagent approved by the FDA for HER-2 detection in colorectal cancer [5].

The IHC operation steps include: dewaxing and hydration of paraffin sections, antigen retrieval, incubation with primary antibody, incubation with secondary antibody, DAB staining, and hematoxylin re-staining. The result interpretation requires observation under a high-power microscope, and reference to the HER-2 scoring standard for breast cancer, which is divided into four grades: 0, 1+, 2+, and 3+. The specific interpretation criteria are as follows:

(1) 0: The tumor cell membranes show no staining at all, or less than 10% of the tumor cell membranes show partial staining;

(2) 1+: More than 10% of the tumor cell membranes show slight/almost imperceptible staining, with only local coloring on the cell membranes;

(3) 2+: More than 10% of the tumor cell membranes show mild/moderate staining, with the cell membranes fully colored;

(4) 3+: More than 10% of the tumor cell membranes show strong/complete circular and uniform staining.

IHC 0 and 1+ are defined as negative and no further testing is required; IHC 3+ is defined as positive,

indicating overexpression of the HER-2 protein; IHC 2+ is considered a suspected positive and further methods such as FISH should be used to verify the gene amplification situation. The American Society for Clinical Pathology (CAP) recommends that at least the primary or metastatic tumor tissues related to the treatment be tested, and a positive cell ratio of no less than 10% can be used to determine HER-2 overexpression.

3.2. FISH

FISH (Fluorescence In Situ Hybridization) is the gold standard method for detecting HER-2 gene amplification. Its principle is to use DNA probes with fluorescent labels, which specifically bind to the HER-2 gene in the tumor cell nucleus and the centromere region of chromosome 17 (CEP17). By observing the signal intensities of both, it is possible to determine whether the HER-2 gene is amplified [6].

The FISH operation steps include: dewaxing and hydration of paraffin sections, baking, DNA denaturation, hybridization, denaturation removal, DAPI re-staining, etc. The commonly used probes include PathVysion, INFORM, PharmDx, etc., all of which are dual-color probes. The red fluorescent dye marking the HER-2 gene is SpectrumOrange, and the green fluorescent dye marking CEP17 is SpectrumGreen.

The result interpretation needs to be conducted under a special fluorescence microscope. For each case, 20 tumor cell nuclei are randomly counted to obtain the signal numbers of HER-2 and CEP17. There are two interpretation indicators:

(1) The ratio of HER-2/CEP17 (HER-2/CEP17 ratio);

(2) The copy number of HER-2.

The interpretation criteria are as follows:

(1) Negative: HER-2/CEP17 ratio < 2.0, and HER-2 copy number < 4.0;

(2) Suspected positive: HER-2/CEP17 ratio < 2.0, but HER-2 copy number ≥ 4.0 and < 6.0;

(3) Positive: HER-2/CEP17 ratio ≥ 2.0 or HER-2 copy number ≥ 6.0.

FISH has the following advantages over IHC:

(1) It can directly reflect the HER-2 gene amplification status, which is more objective and accurate;

(2) The results are stable and not affected by factors such as tissue fixation;

(3) The staining signal can be preserved for a long time, making it convenient for re-reading [7].

However, FISH also has certain limitations, such as complex operation, long cycle, high cost, and high requirements for equipment and technology. In addition, due to genetic alterations such as chromosome 17 polysomy, in a few cases, inconsistent HER-2/CEP17 ratio and HER-2 copy number may occur, and careful interpretation is required.

3.3. Real-time quantitative PCR

Real-time quantitative PCR (RT-qPCR) is a DNA quantification method based on PCR, which can rapidly and sensitively detect the copy number of the HER-2 gene. Unlike IHC and FISH, RT-qPCR does not rely on tumor tissue sections. It can directly extract DNA from fresh tissues, frozen tissues, or paraffin-embedded tissues for detection, and has lower requirements for tissue quantity, especially being suitable for cases with few biopsy tissues or ctDNA detection from peripheral blood [8].

RT-qPCR uses the Taqman probe method, which enables real-time quantitative detection of the amplification products simultaneously during PCR amplification through specific primers and fluorescently labeled probes. Common internal reference genes include ALB, B2M, G6PDH, etc., which can correct for differences in DNA input and PCR efficiency. By comparing the Ct values of the HER-2 gene and the internal reference gene, the

relative copy number of the HER-2 gene can be calculated.

The RT-qPCR operation steps include: DNA extraction, preparation of the reaction system, real-time quantitative PCR amplification, and result analysis. Currently, there are multiple commercial kits available, such as Cobas 4800 HER-2, Therascreen HER-2 ARMS-PCR, etc., which provide standardized detection procedures and result interpretation. Taking Cobas 4800 HER-2 as an example, with a DNA copy number ≥ 6.0 as the positive judgment standard, the consistency with FISH results is as high as 96%.

Compared with IHC and FISH, RT-qPCR has the following advantages:

(1) Simple operation, short cycle, high throughput;

(2) Objective and accurate quantitative results with good repeatability;

(3) High sensitivity, capable of detecting low-copy-number gene amplification [9];

(4) Can utilize paraffin tissue specimens, facilitating retrospective studies;

(5) Can detect ctDNA in peripheral blood, enabling non-invasive dynamic monitoring.

However, RT-qPCR also has certain limitations, such as being susceptible to factors such as PCR inhibitors in the sample, non-tumor cell contamination, etc., which may lead to false-negative results. In addition, RT-qPCR only reflects the copy number of the HER-2 gene and cannot provide information on HER-2 protein expression, which may have a poor correlation with clinical efficacy.

4. Clinical and pathological characteristics of HER-2 positive colorectal cancer

4.1. Tumor location: HER-2 positivity is more common in rectal cancer, with a higher positive rate than in colon cancer

Multiple studies have shown that the positive rate of HER-2 in rectal cancer is higher than that in colon cancer [10]. A study involving 105 patients with colorectal cancer found that the difference in HER-2 positivity between rectal cancer and colon cancer may be related to the different anatomical locations, embryonic developmental origins, and molecular biological characteristics of rectal cancer and colon cancer [11]. Rectal cancer is more susceptible to local microenvironmental influences, such as chronic inflammation and intestinal flora imbalance, which may promote the overexpression of HER-2. Additionally, the prognosis of rectal cancer patients is generally worse than that of colon cancer patients, suggesting that HER-2 may be one of the important factors affecting prognosis.

4.2. Gender: The HER-2 positive rate in females is higher than that in males

Epidemiological data show that the incidence and mortality rates of colorectal cancer exhibit significant gender differences, with female patients having a better prognosis than male patients. Studies have found that the abnormal activation of the mitogen-activated protein kinases (MAPK) signaling pathway plays an important role in the occurrence and development of CRC. In terms of HER-2 expression, the positive rate in female patients is also higher than that in male patients [12]. Gender-related factors such as estrogen may affect the occurrence and development of colorectal cancer by regulating HER-2 expression. In addition, HER-2 positive breast cancer mostly occurs in pre-menopausal women, suggesting that ovarian function may be involved in regulating the HER-2 signaling pathway. Future research is expected to clarify the molecular mechanism of gender differences and provide new ideas for individualized prevention and treatment of colorectal cancer.

4.3. Histological type: HER-2 positivity is more common in mucinous adenocarcinoma, signet ring cell carcinoma and other special types

The distribution of HER-2 positivity varies among different histological types of colorectal cancer. Generally, the positive rate of HER-2 in mucinous adenocarcinoma and signet ring cell carcinoma, etc., is higher than that in ordinary adenocarcinoma. A study involving 60 patients with colorectal cancer found that the expression of COX-2 in all colorectal tissues was determined by immunohistochemistry, and the correlation between COX-2 expression and clinical pathological features was analyzed. The result showed that the COX-2 positive rate in CRC tissues was 46.67%, significantly higher than 11.67% in normal colorectal tissues ($P < 0.05$) [13]. These special types often have a poorer prognosis, suggesting that HER-2 may affect prognosis by promoting the secretion of mucus by tumor cells and their differentiation towards a neuroendocrine direction. In addition, the occurrence of these special types may be related to specific genetic alterations, such as MSI-H, CIMP, etc., and these alterations may also have cross-relationships with the HER-2 signaling pathway, which requires further research to confirm.

4.4. Degree of differentiation: The differentiation degree of HER-2 positive patients is generally poor

Tumor differentiation degree is one of the important indicators for judging the malignancy and prognosis. Multiple studies have found that the tumor differentiation degree of HER-2 positive colorectal cancer patients is worse than that of HER-2 negative patients [14]. Chen Minyang et al. included 80 colorectal cancer patients in their study and showed that the expression level of HER2 was positively correlated with clinical stage, invasion depth, lymph node metastasis, distant metastasis, and pathological features such as differentiation degree. The survival rate of HER-2 positive patients was significantly lower than that of negative patients [15]. Among them, the lower the differentiation degree, the higher the atypia of tumor cells, and the stronger the proliferative and invasive ability. HER-2 activates downstream PI3K/Akt and MAPK signaling pathways to promote cell cycle progression, inhibit apoptosis, and may lead to tumor cell dedifferentiation. In addition, HER-2 can also upregulate the expression of certain stem cell markers, such as CD44 and CD166, enabling tumor cells to acquire stem cell-like characteristics, thereby accelerating tumor progression.

4.5. TNM staging: The positive rate of HER-2 increases with the elevation of T stage, N stage and M stage

The positive rate of HER-2 is closely related to the TNM staging of colorectal cancer. He *et al.* have shown that HER-2 may promote tumor progression by enhancing the invasive and metastatic ability of tumor cells. HER-2 can induce epithelial-mesenchymal transition, enabling tumor cells to exhibit malignant manifestations such as migration and invasion [16]. Moreover, HER-2 can promote tumor angiogenesis, disrupt the basement membrane, and provide a pathway for tumor metastasis and spread. Therefore, it is particularly important to conduct HER-2 testing for advanced patients, and those with positive results can benefit from targeted drugs such as trastuzumab.

4.6. Sites of metastasis: HER-2 positive patients are more prone to liver metastasis, followed by lung metastasis and peritoneal metastasis

HER-2 affects the pattern and sites of metastasis in colorectal cancer. Studies have shown that HER-2 positive patients are more likely to develop liver metastasis. A study involving 126 patients with metastatic colorectal cancer revealed that the expressions of her-2 and VEGF proteins were higher in colorectal cancer, and their expressions were closely related to the growth, invasion and metastasis of colorectal cancer, possibly having a synergistic effect [17]. The combined detection of HER-2 and VEGF expressions in the tissues of colorectal cancer

patients is helpful for evaluating the severity of colorectal cancer and the prognosis of patients, providing certain scientific basis for the early diagnosis and molecular treatment of colorectal cancer in the future [18]. Thus, HER-2 may promote tumor cell metastasis to the liver through various mechanisms, such as upregulating the expression of matrix metalloproteinases, degrading the extracellular matrix; inducing epithelial-mesenchymal transition, enhancing cell motility; promoting tumor angiogenesis, providing pathways for metastasis, etc. In addition, HER-2 positive patients have a higher risk of lung metastasis and peritoneal metastasis, which may be related to the lymphangiogenesis mediated by HER-2 and the changes in adhesion factor expression.

5. Research on HER-2 as a prognostic predictor for colorectal cancer

HER-2 as a prognostic predictor for colorectal cancer holds significant research value and clinical significance. Numerous studies have shown that HER-2 positivity is closely associated with poor prognosis in patients with colorectal cancer, suggesting that HER-2 may be a potential prognostic marker [19]. HER-2 participates in the invasion and metastasis process of colorectal cancer through various mechanisms, such as activating downstream signaling pathways, inducing epithelial-mesenchymal transition, and promoting tumor angiogenesis, ultimately leading to a shorter survival period for patients. Systematic evaluation of HER-2 expression status is of great value in predicting the prognosis of colorectal cancer patients and guiding treatment decisions.

However, there are still some controversies and challenges regarding HER-2 as a prognostic marker for colorectal cancer at present. Firstly, the expression frequency of HER-2 in colorectal cancer is relatively low, with a positive rate of approximately 5%, which to some extent limits its clinical application value [20]; Secondly, different detection methods and interpretation standards lead to significant differences in the positive rate of HER-2, and there is still a lack of unified detection norms; Moreover, the heterogeneity of HER-2 expression may cause false negative results, and the HER-2 status may change during tumor progression, so dynamic monitoring is required.

In addition, HER-2 may also become an important target for the treatment of colorectal cancer. Anti-HER-2 drugs such as trastuzumab have shown good efficacy in the treatment of HER-2 positive breast cancer and gastric cancer, but research in the field of colorectal cancer is still in its infancy. The preliminary results of clinical trials such as HERACLES are encouraging, indicating that trastuzumab combined with chemotherapy or other targeted drugs may bring survival benefits to HER-2 positive, treated advanced colorectal cancer patients. In the future, large-scale, prospective clinical trials are needed to further verify the efficacy and safety of anti-HER-2 treatment in different molecular subtypes and different treatment settings, optimize the dosage regimen and combination strategies, and evaluate its impact on patients' quality of life and medical expenses. In addition, discovering new resistance mechanisms and developing new anti-HER-2 drugs will be the focus of future research.

5.1. Drug treatment targeting HER-2

Trastuzumab is a humanized monoclonal antibody that specifically binds to the extracellular region of HER-2 and blocks the HER-2 signaling pathway, thereby inhibiting the proliferation of tumor cells. A study included 584 patients with HER-2-positive advanced gastric or gastroesophageal junction cancer. The results showed that trastuzumab combined with chemotherapy significantly prolonged the median survival time of patients compared to chemotherapy alone (13.8 months vs. 11.1 months, HR = 0.74, P = 0.0046) [21]. Based on these results, trastuzumab combined with chemotherapy has become the standard first-line treatment for HER-2-positive

advanced gastric cancer.

The HERACLES trial included 27 patients with HER-2-positive metastatic colorectal cancer who received trastuzumab and lapatinib (a small molecule tyrosine kinase inhibitor) in combination therapy. The objective response rate was 30%, the disease control rate was 59%, and the median progression-free survival was 21 weeks. This study confirmed that anti-HER-2 treatment may bring survival benefits to this group of patients [22]. Subsequently, the HERACLES-RESCUE trial used trastuzumab combined with TDM-1 (a trastuzumab-emtansine conjugate) to treat HER-2-positive, RAS/BRAF wild-type metastatic colorectal cancer, and the preliminary results were encouraging.

Furthermore, some new antibody drugs targeting HER-2, such as Pertuzumab and MM-111, as well as various small molecule tyrosine kinase inhibitors like Lapatinib, Neratinib, and Pyrotinib, are currently in the clinical trial stage.

6. Conclusion

In conclusion, HER-2 plays a significant role in the occurrence, development and prognosis of colorectal cancer. HER-2 positivity indicates a poorer prognosis for patients, but it also provides new ideas for targeted therapy for this group of patients. Anti-HER-2 drugs such as trastuzumab have shown good efficacy and safety in the treatment of HER-2-positive advanced colorectal cancer, but more clinical trial data are needed for verification. In the future, with the in-depth study of the molecular mechanism of HER-2, the continuous emergence of new drugs, and the optimization of treatment regimens, HER-2 is expected to become one of the most important therapeutic targets for colorectal cancer. At the same time, how to select patients suitable for anti-HER-2 treatment, early identification and reversal of drug resistance will be the key research directions in the future.

In summary, the role of HER-2 in the diagnosis and treatment of colorectal cancer is increasingly prominent. Only by strengthening basic research, standardizing and popularizing HER-2 testing, actively conducting clinical trials can more patients benefit from it, and thereby improve the overall diagnosis and treatment level and patient survival quality of colorectal cancer. It is believed that in the and near future, HER-2 testing will become an important part of the routine diagnosis of colorectal cancer, and anti-HER-2 drugs will benefit more patients in need.

Disclosure statement

The authors declare no conflict of interest.

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