Hong Kong J Radiol. 2012;15(Suppl):S40-5

REVIEW ARTICLE

Predictive Molecular Biomarkers in Targeted Therapy of Gastric Carcinoma and Metastatic Colorectal Carcinoma

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ABSTRACT

With the recent advancement of molecular targeting therapy, we are witnessing a paradigm shift in the role of pathology laboratories in the management of solid tumours. Pathologists not only continue to provide accurate pathological diagnosis and prognostic information, but also incorporate information about predictive biomarkers in the guidance of individualised treatment. Predictive biomarker study has become increasingly important to clinicians in formulating personalised therapies for their patients. Accurate determination of these predictive biomarkers has become the expected standard of care for cancer patients. Tailoring therapeutic regimens for advanced gastric carcinoma and metastatic colorectal carcinoma demonstrate the inevitable obligations for predicative biomarker study in the overall management of individual patients. In this review, we will discuss the biomarker analysis of HER2 status in advanced gastric carcinoma and KRAS *mutation in metastatic colorectal carcinoma. Issues related to these biomarker determinations in the application of targeted therapy are illustrated.*

Key Words: *Colon; Proto-oncogene proteins; Stomach*

中文摘要

胃腺癌和轉移性結直腸癌的標靶治療中的預測性分子生物標記

羅穎業、杜家輝

隨著分子標靶治療不斷進步，我們對病理學實驗室在腫瘤治療中的角色有著思考模式的轉移。病理學醫生不只繼續提供準確的病理學診斷及預後資料，更能加入預測性生物標記來引導個別的治療方案。預測性生物標記在醫生為病人制定個人化的治療方案中相當重要，其精確的測定已成為癌症病人的護理標準。為晚期胃腺癌和轉移性大腸癌患者度身訂造的治療方案顯示這些預測性生物標記在個別病人的整體護理上不能替代的任務。本文會討論晚期胃腺癌中HER2的生物標記分析，以及轉移性大腸癌中的*KRAS*突變。並探討生物標記在使用標靶治療中有關的問題。

INTRODUCTION

With more in-depth understanding of the molecular alterations in human cancers and the recent advancements in molecular targeted therapy, the landscape for treating patients with solid tumours is rapidly transforming. Hormonal treatment for patients with oestrogen receptor–positive breast cancer is considered to be the first targeted therapy with an

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available predictive biomarker.[1,2] Since such biomarker study is crucial in patient management, a final pathology report of a breast cancer specimen is deemed incomplete without assessment of the status of the oestrogen receptor, progesterone receptor, proliferation index, and HER2 status. Oncologists depend on such information to determine the management plan for an individual patient. It is evident that only a subset of patients with the same cancer type may respond to a particular type of targeted therapy. This underscores the paramount importance of predictive marker analysis in order to identify patients who may benefit from targeted therapy. The recent Trastuzumab for Gastric Cancer (ToGA) trial showed that patients with HER2-positive advanced gastric carcinoma (GCA) and gastroesophageal junction adenocarcinoma (GEJA) will benefit from the addition of the anti-HER2 treatment trastuzumab.[3] HER2-positive status is considered to be a positive predictive biomarker for anti-HER2 targeted therapy. A subset of patients with metastatic colorectal carcinoma (mCRC) may benefit from the anti–epidermal growth factor receptor (EGFR) therapy cetuximab. However, a positive predictive biomarker is not available. In contrast, *KRAS* mutation has been shown to be a negative predicative biomarker for anti-EGFR targeted therapy in patients with mCRC.[4] Currently, assessment of HER2 status in patients with advanced GCA or GEJA and *KRAS* mutation status in patients with mCRC is part of the standard care when patients are being considered for targeted therapies. In this review, we will discuss the issues related to the assessment of HER2 status in advanced GCA and *KRAS* mutation testing in mCRC, with emphasis on the importance of accurate pathological assessment in these biomarker studies.

PATHOLOGICAL ASSESSMENT IN PREDICTIVE BIOMARKER STUDIES IN SOLID TUMOURS

All biomarker studies must be based on accurate pathological diagnosis.[5] Many biomarker studies currently performed are based on biopsy, cytological, or excision specimens. If biomarker studies are anticipated, it is the duty of both the pathologists and the laboratory staff to safeguard the patient samples. When biomarker analysis is required, the pathology report and slides are retrieved for review by a pathologist. The appropriate tissue block or sample is selected based on the quality of the material, tumour cell content, and the nature of the testing platform. Macro- or micro-dissection to isolate and enrich tumour cell content may be required. This is especially important for mutational analysis. For cases in which multiple biomarker studies may be needed, communication with clinical colleagues is necessary to determine whether such studies are better carried out simultaneously or sequentially with prioritisation. If the sample is inadequate or is judged not to be suitable, additional samples for analysis may be required. Timely communication with the treating clinician will be particularly important in this situation. The laboratory procedures should follow the most robust, time-honoured, and widely accepted protocols. The laboratory can choose from commercially available standardised kits or use methods developed in house. In either case, the protocols need to be validated by both internal and external validation processes. Documentation of validation and adherence to internal quality controls and external quality assurance are essential. It is also important for the laboratory to join the relevant accreditation schemes if accreditation of a particular biomarker study is available and a scheme is offered by a recognised accreditation body. These measures help to ensure the reliability of the results being issued by the laboratory. Understandably, timely delivery of this critical information to the treating clinician is important.

HER2 TESTING IN GASTRIC CANCER

Gastric cancer remains one of the leading causes of cancer deaths worldwide and it is the fourth commonest cause of cancer deaths in Hong Kong.[6] Patients often present at an advanced stage and the prognosis is poor.[7] Despite advancements in chemotherapy, the median overall survival is around 10 to 11 months for patients with advanced stage gastric cancer.[8] The ToGA phase III trial had a significant impact on the management of patients with advanced stage GCA.[3] This trial showed that trastuzumab, the monoclonal antibody against human EGFR 2 (HER2), in combination with chemotherapy was beneficial for patients with HER2-positive GCA or GEJA. Among 3665 patients with advanced stage GCA recruited worldwide, 594 (approximately 16%) had HER2-positive adenocarcinoma. Trastuzumab confirmed significant benefits in patients with HER2-positive adenocarcinoma in overall survival, progression-free survival, and overall tumour response rate.[3,9] Trastuzumab is the first targeted therapy approved for treating HER2-positive advanced GCA and GEJA. Data from the ToGA study indicated that HER2 status is a predicative biomarker for trastuzumab targeted therapy. Thus, HER2 testing is not only needed for patients with breast cancer, but also for those with advanced stage GCA.

Accurate determination of HER2 status is crucial. HER2 over-expression or gene amplification has been known to exist in a subset of patients with GCA. The biological significance is not entirely clear, but the amplification of HER2 may be associated with more aggressive behaviour of the cancer.[10] However, there is no standardised assessment of HER2 status for GCA. In the post-hoc exploratory analysis in the ToGA trial, HER2-positive status was defined as immunohistochemistry (IHC) score 3+ or IHC score 2+ with positive fluorescence in-situ hybridisation (FISH).[3,11] For patients with HER2-positive adenocarcinoma, trastuzumab plus chemotherapy versus chemotherapy alone yielded a significant prolongation of median overall survival of 16.0 months versus 11.8 months. Laboratory testing of HER2 status as the predictive biomarker therefore has to follow the standardised guideline. Notably, in the ToGA study, 7.5% of recruited patients with HER2 FISH-positive results, but with IHC 0/1+, did not gain benefit from the addition of trastuzumab.[3,9] Thus, pathologists need to follow the two-step approach in the assessment of HER2 status in GCA, with IHC being performed first, followed by gene amplification study if required. IHC 3+ is regarded as HER2 positive; IHC 0/1+ is regarded as HER2 negative (Figure 1); IHC 2+ is considered as equivocal and gene amplification study is required. If *HER2* gene amplification is found, the tumour is regarded as HER2-positive GCA, otherwise, it is considered HER2-negative GCA.

The HER2 assessment process for GCA is similar to that for breast cancer, but with some important differences. The IHC staining pattern tends to be incomplete basolateral membrane staining in GCA compared with complete membrane staining in breast cancer.[12,13] Pathologists need to be familiar with this difference in

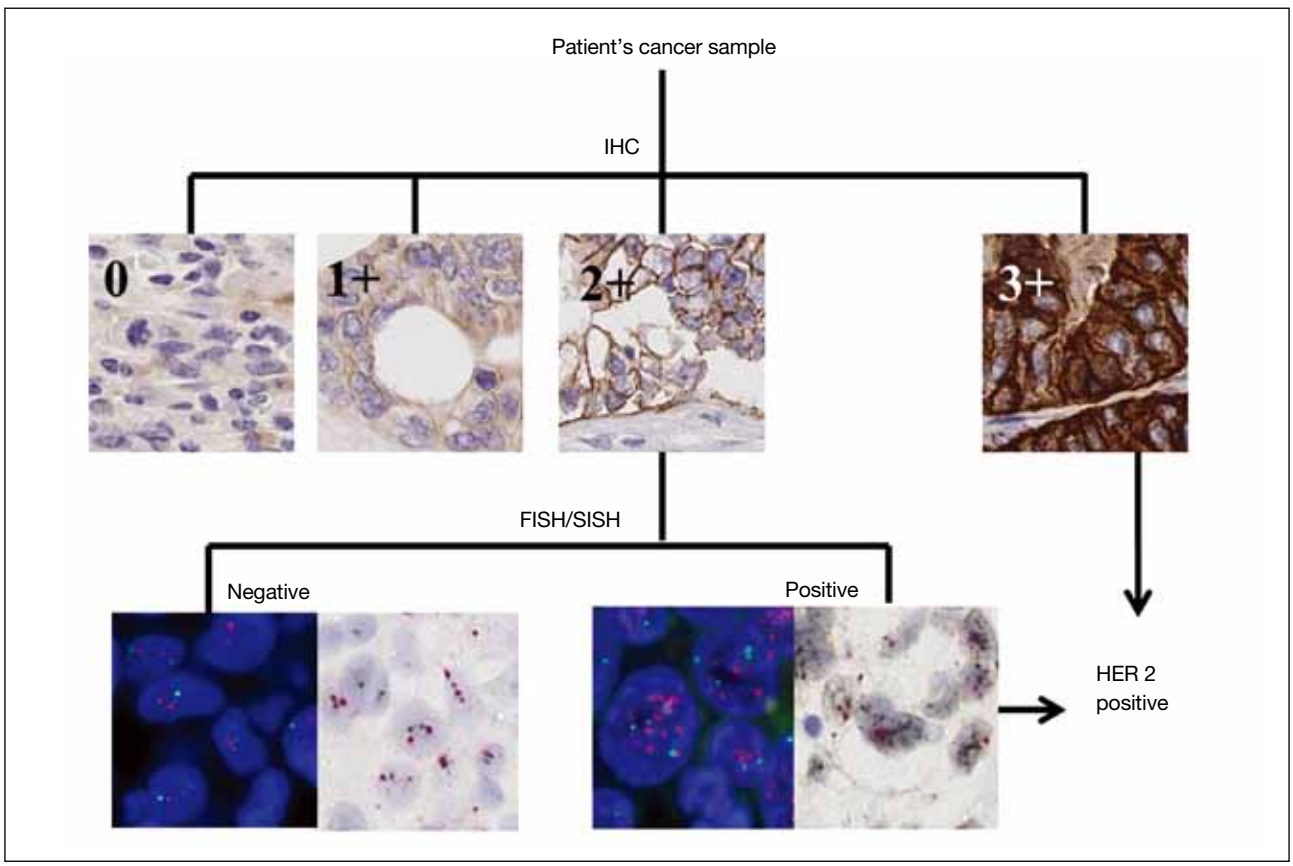

Figure 1. Work flow of HER2 testing in gastric carcinoma. The cancer sample first undergoes immunohistochemical staining (IHC) for HER2. Zero or weak (IHC 1+) staining is designated HER2-negative, while strong staining (IHC 3+) is designated HER2-positive. Equivocal cases (IHC 2+) are further tested for fluorescence in situ hybridisation (FISH; a red signal refers to HER2 probe, a green signal refers to chromosome 17 centromere probe, and nuclei are counterstained in blue) or dual-colour silver in situ hybridisation (SISH; black refers to HER2 signals and red refers to chromosome 17 centromere signals) for *HER2* amplification. IHC2+ and FISH/SISH-positive cancer is also designated as HER2 positive.

Figure 2. Immunohistochemical staining for HER2 in a poorly differentiated adenocarcinoma of the stomach. This example shows a pattern of heterogeneous staining that is fairly commonly encountered. Some tumour cells show strong expression (IHC 3+) while others vary between moderate, weak, and no signals (IHC 2+ to 0).

the staining patterns. More importantly, IHC staining is known to be heterogeneous in GCA, meaning that in HER2-positive GCA, often only a portion of carcinoma cells exhibit IHC 3+ staining or IHC 2+ with positive FISH staining (Figure 2). Data from the ToGA study showed that the HER2-positivity rate for biopsy samples versus excision samples was different. This may also be partly related to the fact that the criteria used for assessing biopsy samples was different from those used for excision specimens. Owing to this heterogeneity, the HER2-positive detection rate may be affected by the number of tissues obtained in endoscopic biopsy. The greater the number of biopsy tissues, the higher the chance of detecting HER2-positive cancer cells in the biopsy. It is preferable to have six pieces of cancer biopsy tissue for assessment. The HER2-positive detection rate could be affected by these pre-analytic factors, and pathologists and clinicians should be aware of this.[12,13]

In the laboratory, a widely tested and validated platform is mandatory for HER2 IHC. Currently, two antibodies have been validated: 4B5 (Ventana Medical Systems, Inc, Tucson, AZ, USA) and A0485 (Dako Denmark A/S, Glostrup, Denmark). These two antibodies are both specific to HER2, but their staining patterns are slightly different. Each individual laboratory needs to validate their methods of choice. For determination of gene amplification, FISH was used in the ToGA trial, but other validated equivalent detection platforms such as dual-colour silver in-situ hybridization (SISH) are also established. For ISH (FISH/SISH) analysis, a *HER2* signal/control signal (HER2/CEP17) ratio of ≥2 is regarded as positive. Due to the heterogeneity of expression of HER2 in gastric cancer cells, it is important to identify the area of interest in which HER2 IHC was shown to be 2+ (equivocal) for the ISH analysis. In this regard, using a bright-field method (SISH or chromogen-based ISH), allowing the simultaneous assessment of morphological features, has its advantages. The bright-field method also enables long-term storage of the slides and, thus, the staining is available for future review. Further efforts to try to combine IHC staining with in-situ hybridisation in the same slide are underway and may facilitate gene amplification analysis.

The criteria being used to assess HER2 status is semi-quantitative and, in part, is unavoidably subjective. In order to minimise inter-laboratory and inter-observer variations, as well as adherence to stringent quality control and assurance, international collaboration to maintain the standard is desirable. A consortium-type of collaboration among Asia-Pacific countries to promote standardisation and share experience was established for assessment of HER2 status in stomach and breast cancers. From the data in the ToGA study, some variations in HER2-positivity rates in GCA among different countries were observed.[3,9] More data from different countries are required to ascertain whether there may be real regional or ethnic differences. The consortium will help to build a landscape of HER2 testing across the Asia-Pacific region. These efforts aim to improve reliability and reproducibility of HER2 testing, increase the accuracy of interpretation by pathologists, and further enhance collaboration between surgeons, gastrointestinal physicians, pathologists, scientists, and oncologists. The ultimate aim is to offer better treatment to patients.

KRAS MUTATION ANALYSES IN COLORECTAL CANCERS

Colorectal cancer (CRC) has become one of the most common cancers worldwide, and is currently the second most common cancer and second commonest cause of cancer deaths in Hong Kong.[6] The prognosis remains poor for patients who present at an advanced stage. It is well known that the pathway involving EGFR is important in the pathogenesis of CRC.[14-16] When the monoclonal antibody against human EGFR-1 became available as a potential targeted therapy, clinical

researchers investigated its application in patients with mCRC.[17-22] Immunohistochemical staining for protein expression of EGFR was initially developed to serve as a potential predictive biomarker.[23] In the Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer (CRYSTAL) study,[24] the laboratory at the Prince of Wales Hospital (PWH) served as the reference laboratory for EGFR IHC testing for the Asia-Pacific region. EGFR IHC was shown not to be useful for predicting responses of CRC patients to the anti-EGFR targeted therapy cetuximab. It was noticed that the status of one of the downstream molecules along the EGFR pathway, *KRAS*, plays a pivotal role in predicting the response of patients with mCRC to cetuximab.[25] Mutation study of *KRAS* is now an essential biomarker test, allowing an informed decision to be made as to whether a patient is eligible for anti-EGFR treatment.

KRAS mutation is a negative predictive biomarker. It is believed that activating *KRAS* mutation may bypass the anti-cancer effect of anti-EGFR that acts upstream of *KRAS*. The benefit of cetuximab is limited to patients with mCRC who harbour wild-type *KRAS*. Detection of *KRAS* mutation is performed in tumour DNA. Assessment of tumour DNA quality and quantity is essential. There is no established standard *KRAS* mutation test that is linked to the original studies. Therefore, different pathology laboratories may employ different methods for detecting *KRAS* mutations. The approach could be a mutation screening technology such as the commonly used direct sequencing method. Alternatively, laboratories may adopt mutation-targeted technology such as the commercially available testing kits or platforms. Mutation screening technologies can detect all known and unknown mutations, while the mutation-targeted technologies only focus on known mutations. Coverage of the mutation spectrum varies depending on the design of the different platforms. The reproducibility, sensitivity, and specificity of each testing protocol may vary. No matter whether the methods are developed in-house or use commercially available standardised kits or platforms, the protocols need to be validated with internal and external validation processes.

Hot-spot mutations in *KRAS* concentrated in codons 12 and 13 are considered to be negative predictors for the anti-EGFR treatment response in patients with mCRC.[26-29] However, recent studies based on retrospective analysis of trial data have suggested that mCRC patients with *KRAS* G13D mutation may have similar response rates to those with *KRAS* wild-type.[30,31] *KRAS* G13D mutation is fairly commonly encountered; among approximately 1500 CRC samples submitted to the laboratory at the PWH for *KRAS* testing, approximately 45% showed *KRAS* mutations and approximately 19% of the *KRAS* mutation–positive samples had G13D mutations. Apart from codons 12 and 13 mutations, there was also evidence to suggest that *KRAS* mutation in codons 61 and 146 may also confer resistance to anti-EGFR targeted therapy in mCRC.[32] From the PWH laboratory data, *KRAS* mutations of codons 61 and 146 account for approximately 5% of the *KRAS* mutation–positive CRC samples. In addition, rare, or even novel, mutations for which no previous data are available for reference have been encountered. As data are accumulated, it may be possible to understand the spectrum of *KRAS* mutations in CRC and the different implications for treatment responses. In facilitating this goal, it is preferable to record the complete information of specific types of mutations in the molecular biomarker reports.

CONCLUSIONS

We are witnessing a paradigm shift in the role of the pathology laboratory in the management of patients with solid tumours. Providing an accurate pathological diagnosis remains the cornerstone of prognostic and predictive biomarker studies. With the rapid advances in targeted therapy, the accurate determination of predictive biomarkers has become the expected standard of patient care. The research showing HER2-positive status in GCA as a positive predictive biomarker and *KRAS* mutation in mCRC as a negative predictive biomarker illustrates the importance and potential issues in biomarker determination. Close collaboration among different health care professionals, including oncologists, pathologists, and scientists, is of paramount importance in the treatment of patients with solid tumours. Understanding the indications, interpretations, and limitations of each of the biomarker studies is crucial to patient management. Pathologists and pathology / molecular laboratories need to stand up to the challenges of quickly evolving knowledge in cancer biology, emerging targeted therapies, biomarker development, and related clinical trials. Laboratory practices need to be structured in such a way that they embrace the ever-changing advancements in technology while retaining the time-honoured diagnostic art in routine practice.

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