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Roles of pathologists in molecular targeted cancer therapy

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Abstract

Molecular targeted cancer therapy (MTCT) is the "personalized" or "individualized" approaches toward cancer which targets the particular molecular or genetic changes, *i.e.* over-expression of molecules, and genetic amplification, mutations and translocations. MTCT is generally composed of two mechanisms, (1) humanized monoclonal antibodies (hMAB) and (2) tyrosine kinase inhibitors (TKI). Somatostatin analogue (SA) is the unique situation for the therapy of neuroendocrine tumors (NETs) which possess somatostatin receptor (SSTR). The cancers which are benefited by MTCT have been increased and will be increased to cover wide varieties of cancers. Good examples are (1) trastuzumab, hMAB against HER2 in breast cancers with HER2 over-expression and amplification, (2) imatinib, TKI, for gastrointestinal stromal tumors (GISTs) with c-kit mutation, (3) gefitinib, TKI, for lung adenocarcinoma with EGFR mutation. The drug effects have been reported to be associated with these molecular and genetic changes. It should be particularly emphasized to treat the patients with corresponding targeted molecular changes. These molecular and genetic analysis should be performed! On the right areas of the cancers, ample amount of viable cancer cells, where the major roles of pathologists are lied. This introductory review of MTCT describes more details of each MTCT.

Introduction

Since the successful and wide use of the anti-HER2 humanized monoclonal antibody, trastuzumab, for treatment of breast cancers, many therapeutic approaches using inhibitors against target molecules in various cancers have actually been in clinical use. Molecular targeted therapy is mainly composed of (1) humanized monoclonal antibodies or (2) small molecule tyrosine kinase inhibitors (TKIs). Current clinically available molecular targeted therapy is listed in Table 1. The humanized monoclonal antibodies are designated as '…mab' and TKIs are designated as '…nib', thereby allowing us to recognize the pharmacological nature of the therapy by their names. In order to select the cancer patients who are expected to respond to the therapy, it is essential for pathologists to detect the appropriate target molecules. The alterations of the target molecules are (1) protein increase/overexpression or (2) gene increase/amplification or (3) gene mutations.

What is molecular targeted cancer therapy (MTCT)?

MTCT can be defined as the therapy which targets specific alterations of proteins or genes and suppresses proliferation and spread of cancers. In contrast, chemotherapy usually targets DNA synthesis and suppresses cancers in a rather non-specific manner, thus even disrupts normal (non-neoplastic) cells. MTCT can be effective for particular group(s) of cancers which express the molecular targets, and does not affect normal cells. The MTCT is important to treat the particular patients who are expected to respond, to keep cost-effectiveness and to avoid the adverse reactions, if any, of the therapy.

Currently, MTCT has been approved by FDA for breast cancers, lung cancers, gastro-intestinal stromal tumours (GISTs), chronic

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Table 1 Types of molecular targeted therapy and corresponding target molecules

Humanized monoclonal antibodies	
Trastuzumab (Herceptin)	HER2 (EGFR2)
Retuximab (Retaxan)	CD20
Bevacizumab (Avastin)	VEGF
Cetuximab (Erbitux)	EGFR1
TKIs	
Imatinib (Glivec)	KIT
Gefitinib (Iressa)	EGFR1
Erlotinib (Tarceva)	EGFR1
Dasatinib Nilotinib Sunitinib	EGFR1
Lapatinib	EGFR1 and EGFR2
Transduction signal inhitors (TSI)	
Everlimus (Afinitor)	mTOR
Hormone analogue	
Octoreotide (Sandostatin)	STTR

myelogenous leukaemias (CML), colorectal cancers (CRCs) and renal cell carcinomas (RCCs), and new therapies are frequently being approved.

Molecular targeted cancer therapy and target molecules

MTCT is composed of humanized monoclonal antibodies (hMABs) or TKIs. The hMABs and TKIs with corresponding targeted molecules are listed in Table 1. As shown previously, alterations of the target molecules include increase in protein levels (overexpression), increase in gene copies (amplification) and gene alterations (mutations). For the overexpression of proteins, not only the increase in protein levels is related to the therapeutic response, but also the activation status, *via* post-translational modifications such as phosphorylation.

As clearly depicted in Table 1 and Fig. 1, most of the target molecules are cell membrane-associated proteins or corresponding genes. These receptors include EGFR1 (EGFR), EGFR2 (HER2), c-kit (KIT) and PDGFR. Recently, the proteins related to the activated signal transduction pathway have been approved as molecular targets, which include members of the mTOR/AKT signalling pathway. These proteins are dependent on the activated membrane receptors or are themselves activated constitutively as illustrated in Fig. 1. In addition to these proteins, somatostatin analogues, octreotide and lanreotide have been used in the patients with neuroendocrine tumours or carcinomas such as pituitary adenomas and gastro-entero-pancreatic neuroendocrine tumours (GEPNETs). The therapeutic response is expected when the tumours express somatostatin receptors, particularly SSTR2a.

Detection of target molecules and therapeutic response

It has been widely known that the expression of molecular targets is related to the response of the therapy. Currently approved MTCTs and molecular targets are described briefly.

EGFR1/EGFR2 (HER2): The EGFR family is composed of four subtypes, EGFR1, EGFR2, EGFR3 and EGFR4, which are transmembrane proteins. They form heterodimers which activate tyrosine kinases (TKs). EGFR1 and EGFR2 (HER2) are currently the only targets of molecular therapy. HER2 was the first target molecule which showed a response to the therapy in advanced breast cancer. This stimulated the subsequent various MTCTs. Resistance to the therapy has been known to occur by developing second and third mutations, such as EGFR and KIT in lung cancers and GISTs, respectively. These changes require further and additional molecular targeted therapy.

EGFR2 (HER2)

Trastuzumab, a humanized monoclonal antibody against HER2, has been effective against breast cancers with HER2 overexpression and gene amplification, the mode of action of which is related to suppression of metastases and even the incidence of recurrent tumours. Thus, it is recommended that the HER2 test should be done for advanced as well as early diseases. The ASCO/CAP guidelines have been implemented in order to validate the performance and interpretation of HER2 analyses in breast cancers [1]. With regard to techniques, *in situ* hybridization (ISH) for detection of gene alterations has been performed with fluorescence ISH (FISH), but recently, bright field techniques, such as chromogenic ISH (CISH) and silver ISH (SISH), have also been applied. Polysomy is a major problem in interpreting HER2 FISH results [2]. Gastric cancers are the next target of trastuzumab therapy, and the detection of HER2 changes are analysed by the same techniques as for breast cancers. However, it has been pointed out that gastric cancers show more heterogeneity in HER2 expression (personal communication).

EGFR (EGFR1)

EGFR is another transmembrane protein that belongs to the EGFR family. In lung cancers, the mutations at exons 20 and 22 are associated with a positive response to Gefitinib, a TKI [3–5]. PCR followed by direct DNA sequencing and FISH are the

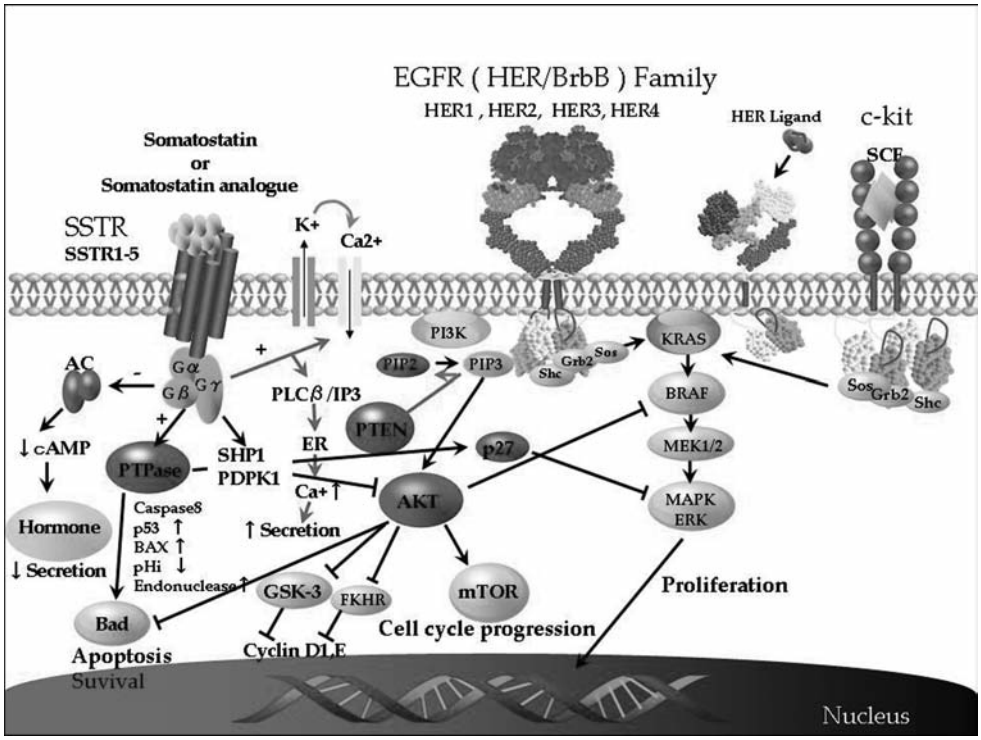

Fig. 1 Schematic illustration of molecular target therapy.

techniques routinely performed in the laboratory for analysis of this gene. Hsitological observation of the lung cancer is of value, because it is known that squamous cell carcinoma lacks EGFR mutation and mucinous carcinoma is known to have KARS mutation. Recently, the use of mutation-specific monoclonal anti-EGFR antibody for analysis of specific cancers has been reported [6]. Further, EGFR amplification has been identified in glioblastoma multiforme [7].

Recently, cetuximab, a humanized monoclonal antibody against EGFR, has been approved for treatment of CRCs. Cetuximab is given to the patients when the tumour is positive for EGFR by immunohistochemistry [8–11] for which a staining kit (PharmDx DAKO Cytomation Carpinteria, CA, USA) is now commercially available. However, mutation in the KRAS gene is a key element related to poor response to cetuximab [12, 13].

c-kit (CD117)

KIT is a transmembrane protein which is also related to TKs. Diagnosis of GIST is made based on the combination of morphology and immunohistochemical positivity for CD34, vimentin and c-kit. Thus, Imatinib, an anti-c-kit TKI, is often used to treat GIST and the GIST patients with mutations on exon 11 respond better to Imatinib. However, mutation on exon 9 is often associated with duodenal GISTs and a poor response to Imatinib, which frequently contributes to a worse outcome [14–16].

Bcr/abl

The translocation of bcr/abl is related to activation of TKs in CML, which can be treated with TKIs such as Imatinib and more recently Nilotinib [17].

Multiple TKIs

The tumours which are activated by multiple TKs and the Imatinib-resistant GISTs with secondary mutations may respond better to the drugs which inhibit multiple TKs, such as Soratinib and Sunitinib. Currently, these multi-targeted TKIs are being used for the treatment of RCC, while Lapatinib, which inhibits both HER2-related TKI and EGFR-related TKI, has been used against Trastuzumab-resistant breast cancers [18, 19].

AKT/mTOR

Growth factor receptor-related intracellular signals are composed of two components, PTEN/AKT/mTOR and RAS/Raf/Erk1/2. AKT and mTOR have been reported to be activated by phosphorylation in various cancers, including ovarian clear cell carcinoma [20], gastric cancer [21] and triple negative breast cancers [22]. In the tumours with phosphorylated(p)-mTOR, for example RCCs, mTOR inhibitors such as Everolimus can inhibit cell growth and has been under clinical trial for practical use [23–26].

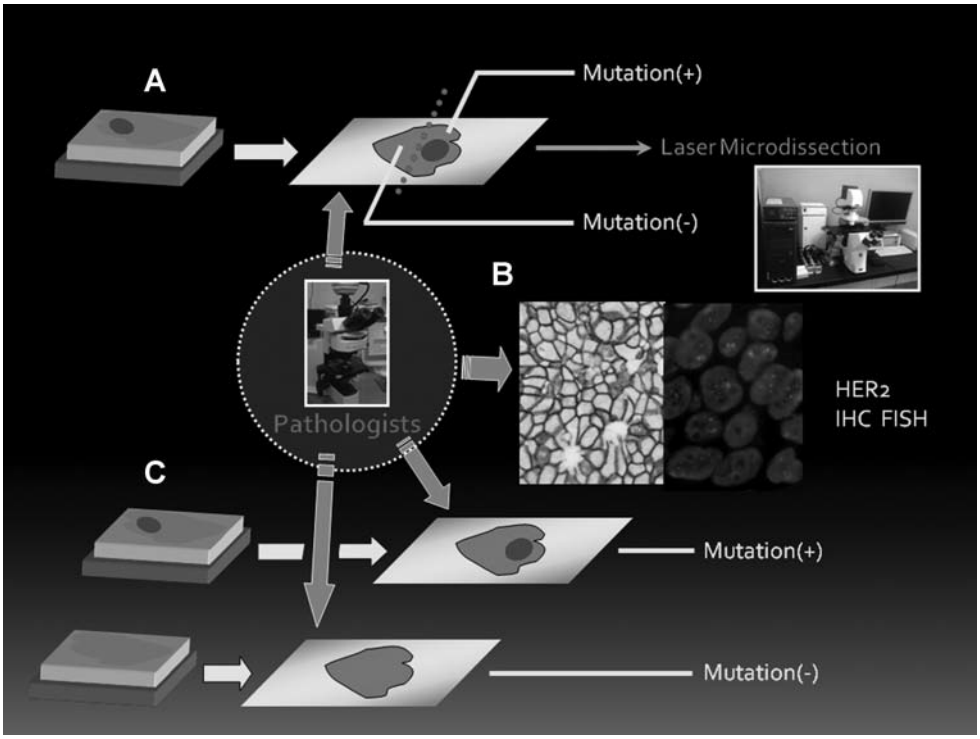

Fig. 2 Illustration of the roles of pathologists. Pathologists are the medical professors who are essential in performing proper molecular tests. The pathologists (**A**) make sure the tests are done on cancer cells in tissue sections, (**B**) perform and interpret molecular morphology, IHC and FISH and (**C**) select the appropriate paraffin blocks for the case. The pathologists also are expected to recommend the most appropriate test method.

Somatostatin receptors

Somatostatin receptors (SSTRs) are composed of five subtypes, SSTR1, 2a, 3, 4 and 5. These SSTRs are expressed in various cell types, and SSTR2a has been shown in the endocrine organs and systemic neuroendocrine cells from which endocrine tumours and neuroendocrine carcinomas are derived, respectively [27–31]. Current therapy is done by synthetic octreotide which binds to SSTR2a more intensely and has been used for the treatment of pituitary adenomas and GEPNETs. Therefore, the detection of SSTR2a by immunohistochemistry is important to anticipate the clinical response of neuroendocrine tumours and carcinomas [32, 33].

Roles of pathologists in molecular targeted cancer therapy

Pathologists use various analytical methods for the detection of appropriate target molecules for MTCT. The following histological and molecular pathological techniques have been used:

1. Immunohistochemistry
2. ISH
3. FISH, CISH, SISH
4. PCR and mutational analysis

In addition to FISH, recently developed CISH and SISH are particularly practical because they can be analysed by ordinary light microscopy, not requiring fluorescent microscopy.

For the appropriate performance of the above tests, pre-analytical (materials handling), analytical (immunohistochemistry and other methodologies) and post-analytical (interpretation and reporting) skills are very important. The roles of pathologists are essential in order to perform these tests with adequate quality control.It should be emphasized that the pathologists are responsible to confirm that the molecular and genetic tests are performed on the right specmen, *i.e.* sections selected to contain ample and viable cancer tissues (cells) (Fig. 2).

Summary

This introduction for the series of the MTCT series is designed to give an overview of the current direction of this field and outlines the roles of pathologists in determining the target molecules for the most appropriate management of patient care.

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