Immunohistochemistry and genomic pathology for molecular targeted cancer therapy

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Recently, one of the major advances in cancer treatment is molecular target therapy which includes several major methods, (1) humanized monoclonal antibodies, (2) tyrosine kinase inhibitors, (3) signal transduction inhibitors, (4) hormonal analogues. The prediction of therapeutic effects depends on the detection of target molecules. Typical examples are (1) detection of HER2 gene over-expression and amplification in breast and gastric cancers, (2) mutations in several genes, i.e. EGFR for lung cancer, KRAS gene in lung and colo-rectal cancers, KIT gene in gastro-intestinal stromal tumor(GIST), (3) detection of activated transduction signals for renal cell carcinoma(RCC) and neuroendocrine tumors(NET), (4) hormone receptors in neuroendocrine tumors(NET).

This lecture outlines the contribution of immunohistochemistry and molecular pathology in molecular targeted cancer therapy and highlights the contribution of immunohistochemistry to predict the therapeutic effects.

**Molecular targeted therapy**

1. Humanized monoclonal antibodies
   - Trastuzumab (Herceptin) HER2 (EGFR2) BC GC
   - Retuximab (Retaxan) CD20 Lymphoma
   - Bevacizumab (Avastin) VEGF CRC
   - Cetuximab (Erbitux) EGFR1 CRC

2. Kinase inhibitors (TKI)
   - Imatinib (Glivec) KIT GIST
   - Gefitinib (Iressa) EGFR1 Lung carcinoma
   - Erlotinib (Tarceva) EGFR1 Lung carcinoma
   - Dasatinib Nilotinib Sunitinib EGFR1
   - Lapatinib (Tykerb) EGFR1 and EGFR2
   - Crizotinib (Xalkori) ELM4-ALK fusion gene Lung carcinoma
   - BRAF mutation thyroid

3. Signal transduction inhibitors (STI)
   - Everolimus (Afinitor) mTOR RCC PNET

4. Hormone analog
   - Octreotide (Sandostatin) STTR NET

**Summary of immunohistochemical and genetic tests**

**For molecular targeted cancer therapy**

Breast and gastric cancer

HER2 IHC positive orISH positive \(\rightarrow\) Trastuzumab
Lung adenocarcinoma
   EGFR mutated & KRAS wild type $\rightarrow$ gefitinib
   EGFR non-mutated & ALK translocation $\rightarrow$ crizotinib

Colo-rectal cancer
   EGFR positive, KRAS wild & BRAF wild $\rightarrow$ cetuximab

GIST
   KIT exon 11 mutated $\rightarrow$ imatinib

NET
   SSTR2a overexpressed $\rightarrow$ somatostatin analogue

1. HER2 gene over-expression and amplification in breast cancer (BC) and gastric cancers (GC)
   It has been well known that humanized monoclonal antibody trastuzumab now is approved and used for metastatic BC (approved 2001 in Japan), adjuvant therapy (2008 approved in Japan), and neoadjuvant, therapy (approved 2011 in Japan) for the breast cancer. Current diagram in most institutions in Japan is to start IHC first and followed by genetic tests for equivocal case.


   The HER2-testing algorithm for breast cancer. CISH, Chromogenic in situ hybridization; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.
   * FISH/SICH and DISH
This algorithm has been modified by ASCO/CAP in 2007. In 2011, Trastuzumab has been approved for GC Methodology: For IHC, polyclonal antibody, HercepTest®(DAKO) or monoclonal antibody 4B5(Roche, Ventana) are used. For genetic test, in situ hybridization(ISH) is the method of choice. FISH is the first method, but now CISH and further DISH are approved and used for the evaluation of HER2 gene amplification. ISH with bright field would be superior to FISH on the particular tumors with HER2 heterogeneity, such as gastric cancer.

2. Mutations of EGFR, KRAS, BRAF, KIT in lung cancers, colo-rectal cancers, GISTs

Gene mutation is another test to predict the therapeutic effects of molecular target therapy. EGFR mutation is essential genetic change for the effective therapy of lung adenocarcinoma by gefitinib

Lung cancers

It has been very well known that the therapeutic effects of gefitinib are correlated with the mutation of EGFR gene, (1) point mutation of Exon 21 L858R and (2) deletion mutation of Exon 19. 746-750. Both mutations account for approximately 90% of all EGFR mutations(FIG). The mutations are most frequently occurring in adenocarcinoma, more often in Asian women and never smokers. And I have been also known that immunohistochemical staining for EGFR is not related to the therapeutic effects.

If the lung cancers show mutation in KRAS gene and BRAF gene, it has been suggested that gefitinib is not effective even though the tumor cells exhibit EGFR mutation.

Screening a panel of 340 paraffin-embedded NSCLC tumor samples with these antibodies showed that the sensitivity of the immunohistochemistry assay is 92%, with a specificity of 99% as compared with direct and mass spectrometry-based DNA sequencing. This simple assay for the election of EGFR mutations in diagnostic human tissues provides a rapid, sensitive, specific, and cost-effective method to identify lung cancer patients responsive to EGFR-based therapies (Jian Yu et al. 2009)

Furthermore, EGFR mutation and EML4-ALK translocation are mutually exclusive. Therefore, if the tumor cells are negative for EGFR mutation, we have to examine the possible EML4-ALK translocation. It is well known that immunohistochemical staining for ALK correlates with EML4-ALK translocation. By FISH, EML4-ALK translocation can be identified as “break apart” mutation. This translocation can occur with mutual exclusion of EGFR & KRAS mutation.

Recently, EGFR mutation-specific monoclonal antibodies have been available to detect mutations of Exon 21 L858R and Exon 19 del746-750. Both antibodies show high specificity and sensitivity. The mutation-specific antibodies are suggested to be used as prescreening for gefitinib therapy.
Colo-rectal cancers

For the metastatic colorectal cancers, humanized anti-EGFR monoclonal antibody, cetuximab, has been used as a therapy if the colo-rectal cancers (CRCs) cells are immunohistochemically positive for EGFR. It has been known that the cetuximab therapy is not effective when the tumor cells contain KRAS mutation or BRAF mutation. So cetuximab therapy should be given to the patients with wild KRAS or BRAF genes.

KRAS mutational analysis is mandatory for appropriate cetuximab therapy. The detection methods include PCR direct sequencing or Kit: Scorpion-ARMS (Roche Diagnostics). It is essential to perform mutational analysis on the tissue sections which contain adequate carcinoma. This requires the practical role of pathologists.

The following is the manufacturer’s package insert for the colo-rectal cancers:

Erbitux® is an epidermal growth factor receptor (EGFR) antagonist indicated for treatment of:

**Colorectal Cancer**

**K-Ras mutation-negative (wild-type), EGFR-expressing, metastatic colorectal cancer as determined by FDA-approved tests**

- In combination with POLFIRI for first-line treatment,
- In combination with irinotecan in patients who are refractory to irinotecan-based chemotherapy,
- As a single agent in patients who have failed oxaliplatin- and irinotecan-based chemotherapy or who are intolerant to irinotecan
Limitation of Use: Erbitux (cetuximab) is not indicated for treatment of K-Ras mutation-positive colorectal cancer.

Gastrointestinal stromal tumor (GIST)
GISTs are the most common mesenchymal neoplasms of the GI tract; Imatinib is a tyrosine kinase inhibitor (TKI) when the tumor cells have KIT and PDGFRα active mutations. The first mutation is thought to be oncogenic and the second mutation is related drug resistance.
KIT mutation in Exon 11 is most common and the tumors with its deletion are more aggressive. Exon 11 mutation correlates well with the imatinib therapy. The GISTs with Exon 9 mutation occur more in the intestinal tract and less sensitive for imatinib.
PDGFRα mutation occurs most often in Exon 18 and resistant to imatinib therapy.
The secondary mutations which are related to imatinib resistance occur in Exon 13 and 17. The GISTs with the mutation in Exon 13 and 17 are responsive to sunitinib.
Thus, mutational analysis in KIT and PDGFRα genes is essential for the therapeutic predictive factor for therapy.

Nishida T et al. Gastric Cancer (2009) 12: 175–188

Primary mutations
3. **Somatostatin receptor SSTR2a in NETs**

It has been well known that somatostatin receptor (SSTR) is composed of five subtypes, SSTR1, SSTR2a, SSTR3, SSTR4 and SSTR5. All SSTRs are localized on the cell membrane. The neuroendocrine tumors which express SSTR2a are expected to respond to the somatostatin analog therapy, Octreotide.

Recent classification of neuroendocrine neoplasms (NEN) are composed of neuroendocrine tumor (NET) G1 and G2 and neuroendocrine carcinoma (NEC) and mixed adenoneuroendocrine carcinoma (MANEC). To differentiate NET G1, NET G2 and NEC, proliferative markers, mitotic indices and Ki67 labeling indices are used.
NET G1 and NET G2 more frequently express SSTR2a and are expected to respond to SA therapy. In our experience, approximately 60% of NETs are positive for SSTR2a and about 70% of them are responsive to SA therapy including stable disease condition.

Recently, mTOR inhibitor, Everolimus, has been approved for the therapy of the pancreatic NETs. Immunohistochemical staining for p-mTOR appears to be correlated to the therapeutic response. Volante et al. 2007 defined the interpretation of SSTR2a according to the pattern of staining to score 0, 1 2 and 3. Score 2 and 3 are membranous staining are interpreted as “positive”.

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Biotherapy  SSTR

Somatostatin receptor detection for the therapy of somatostatin analogues

**Somatostatin receptors (SSTR)**

SSTR1  SSTR2a  SSTR3  SSTR4  SSTR5

Immunohistochemical detection of SSTR2a

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Schematic illustration of somatostatin receptor type 2A immunohistochemical scoring system and its concordance rate with 111In-pentetreotide in vivo imaging.
References

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Astrid Hirschmann,1 Tiffany Ann Lamb, PhD,2 Georges Marchal,3 Mary Padilla, MD,4 and Joachim Diebold, MD1 Simultaneous Analysis of HER2 Gene and Protein on a Single Slide Facilitates HER2 Testing of Breast and Gastric Carcinomas Am J Clin Pathol 2012;138:837-844


2. Lung cancer and EGFR and ALK


Jian Yu et al. Mutation-Specific Antibodies for the Detection of EGFR Mutations in Non-Small-Cell Lung Cancer Published Online First on April 14, 2009 as 10.1158/1078-0432.CCR-08-2739


3. Colo-rectal cancer and KRAS


4. Gastrointestinal stromal tumor (GIST)


Wong NA, Shelley-Fraser G. Specificity of DOG1 (K9 clone) and protein kinase C theta (clone 27) as immunohistochemical markers of gastrointestinal stromal tumour. Histopathology. 2010 Aug;57(2):250-8.


5. Neuroendocrine tumors and SSTR

