Part I Prediction of Tumor Behavior

1 Beyond Typing and Grading: Target Analysis in Individualized Therapy as a New Challenge for Tumour Pathology

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Abstract

In order to bring about its beneficial effects in oncology, targeted therapy depends on accurate target analysis. Whether cells of a tumour will be sensitive to a specific treatment is predicted by the detection of appropriate targets in cancer tissue by immunohistochemistry or molecular methods. In most instances this is performed by histopathologists. Reliability and reproducibility of tissue-based target analysis in histopathology require novel measures of quality assurance by internal and external controls. As a model for external quality assurance in targeted therapy an annual inter-laboratory trial has been set up in Germany applying tissue arrays with up to 60 mammary cancer samples which are tested by participants for expression of HER2/neu and steroid hormone receptors.

For decades, clinical cancer research was focussed on the study of empirical combinations of non-specific cytotoxic drugs. In recent years oncology has been witnessing a revolution sparked by targeted therapies, notably the chimeric monoclonal antibodies against surface molecules such as CD20 or epidermal growth factor receptor. Meanwhile almost all patients suffering from B cell lymphomas are treated with this mode of therapy (Cheson 2006). How does this revolution of therapy interfere with the classical function of histopathology to classify and to grade malignant neoplasm? Will morphological categories be replaced by a list or profile of markers which constitute potential targets for therapy? This will certainly not be the case, although the biological significance of lymphoma classification has to be reconsidered against the background of treatment response, which will potentially be more relevant than the spontaneous course of disease.

Whereas the task of typing and grading will still form the indispensable basis of cancer therapy, additional challenges with regard to reliability and reproducibility of target identification are awaiting modern pathology. Cancer ceases to be invincible—as has happened to antique heroes before—once its concealed vulnerable spot is known to the opponent. Already in the ancient myth, it required a person to uncover the secret and tell Paris to aim at Achilles‘ heel instead of his armpit (which would have been appropriate to wound Ajax). Similarly, there is good reason to believe that in the case of cancer the pathologist will be the one to reveal the secret and to guide the strike to the appropriate spot.

Targeted therapy requires the correct detection and identification of the potential molecule which might be suitable to interrupt the sustained proliferation of tumours (Savage and Antman 2002). For tissue-based analysis, immunohistochemistry provides a widely used tool to investigate cell-specific expression and to discriminate tumour cells from bystander cells. Specific mutations of potential target genes which lead to malignant transformation are best detected by polymerase chain reaction (PCR) or fluorescence in situ hybridization (FISH). Like immunohistochemistry, both methods can be applied to formalin-fixed and paraffin-embedded tissues.

In principle there are three different settings in which target molecules are detected in cancer cells:

1. Cancer cells retain some physiological properties of the normal counterpart and express

tissue-specific differentiation markers which may be used as targets (e.g. CD20, CD52, EGF-R, steroid hormone receptors).
2. Cancer cells overexpress certain molecules which could serve as targets, whereby the enhanced amount of protein provides the decisive alteration rather than the type of genetic modification (Her2/neu, c-kit, VEGF).
3. Genetic alterations induce the formation of novel non-physiological proteins which can be specifically targeted (c-kit, bcr-abl, FIP1L1-PDGFRα, EGF-R).

Whereas in the first two categories immunohistochemistry represents the method of choice, PCR and FISH dominate in the third. Potential target molecules and their detection are listed in Table 1.1.

There is little doubt that this list will grow and that pathologists will be confronted with the expanding task to specifically guide therapy by the detection of target molecules.

Are pathologists prepared to take over the task to guide targeted therapy, and are their methods reliable enough to prove the presence or absence of an appropriate target on a cancer cell? This is still an open question and a major cause for uncertainty with regard to modern therapies. In particular, quantitative parameters might be insufficiently reproducible. Principally, there are two ways to cope with this problem: centralization of diagnostics or standardization of diagnostics in a multicentric setting. In Germany pathologists have decided to opt for the second alternative; consequently, nation-wide trials for tissue-based markers in breast cancer have been set up (Rudiger et al. 2002, 2003).

Evaluation of potential targets for therapy is not a completely new challenge for pathologists, because immunohistochemical detection of oestrogen and progesterone expression in breast cancer has been used instead of the more inaccurate biochemical extract-based method of detection for more than two decades already. Therefore, the immunohistochemical detection of steroid hormone receptors has become the model system for instigating a new kind of interlaboratory trial. In these trials, tissue arrays are used for testing the reproducibility of oestrogen- and progesterone receptor assessment (Fig. 1.1;

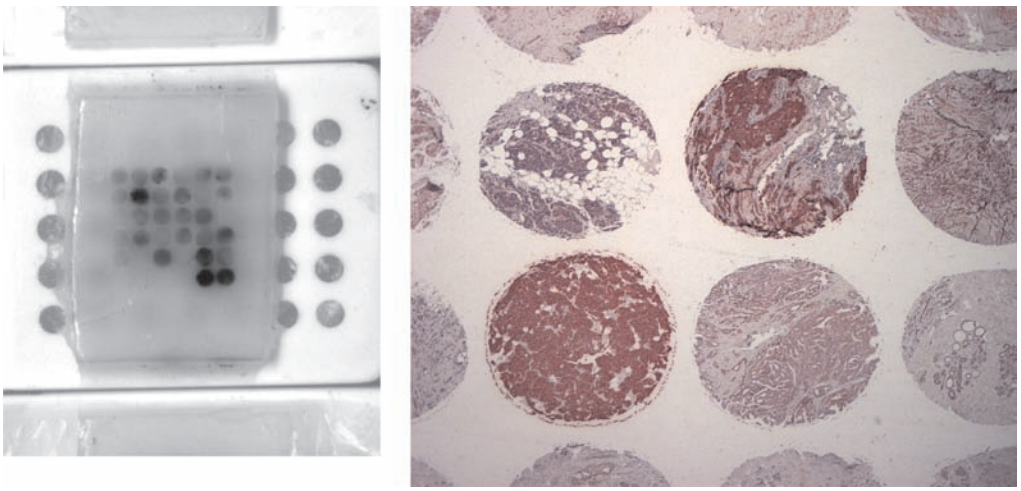

Fig. 1.1 Paraffin block of a tissue array (*left*) which is used in the immunohistochemical quality assurance trial. Thirty different tumour samples with defined target expression are assembled in one slide which has been stained for cytokeratin (*right*). Up to 200 slides can be produced from one tissue array assuring that all participants in the trial obtain almost identical material and that results among different laboratories become comparable. In the quality network of the German Society for Pathology and the Berufsverband Deutscher Pathologen („QuIP", www.ringversuch.de; http://www99.mh-hannover.de/institute/pathologie/dgp) quality assurance trials based on tissue arrays have been set up for different target molecules (ER, PR, Her2, c-kit)

Table 1.1 Potential targets for specific therapy

	Cancer type	Molecule; mode of activation	Detection	Specific drug
Steroid hormone receptors	Breast	Nuclear receptor; unknown	Immunohistochemistry	Tamoxifen, inhibitors of aromatase
Her2/neu	Breast, lung, thymus	Tyrosine kinase of membrane receptor type; gene amplification	Immunohistochemistry, FISH	Humanized monoclonal antibody against Her2 (Trastuzumab)
Epidermal growth factor receptor (EGF-R)	Colon, lung, glioma	Tyrosine kinase of membrane receptor type; gene amplification, point mutation	Immunohistochemistry	Humanized monoclonal antibody, gefitinib, erlotinib
c-kit (stem cell factor receptor)	Gastrointestinal stroma tumours, mastocytosis	Tyrosine kinase; point mutation	Immunohistochemistry, gene sequencing	Imatinib (not all types of mutation)
Bcr-abl	CML, ALL	Tyrosine kinase; fusion gene by chromosomal translocation	FISH, PCR	Imatinib
Platelet-derived growth factor receptor PDGFRα, -β	Gastrointestinal stroma tumours, chronic eosinophilic leukaemia	Tyrosine kinase; fusion gene by chromosomal translocation, point mutation	PCR, Immunohistochemistry	Imatinib
Vascular endothelial growth factor (VEGF)	Various types (e.g. colon)	Soluble cytokine; paracrine production	None	Humanized monoclonal antibody against VEGF (bevacizumab)
Vascular endothelial growth factor receptor VEGFR1–3	Various types (e.g. AML, kidney, glioma)	Tyrosine kinase of membrane receptor type; paracrine activation, amplification	Immunohistochemistry	Su11248, sunitinib
CD20	Normal and neoplastic B lymphocytes	Membrane glycoprotein; unknown function	Immunohistochemistry	Humanized monoclonal antibody (rituximab)
CD52	Normal and neoplastic T lymphocytes	GPI-anchored antigen	Immunohistochemistry	Humanized monoclonal antibody (alemtuzumab, Campath)

Mengel et al. 2003). The trials are conducted annually with up to 180 participating laboratories in Germany (http://www.ringversuch.de; http://www99.mh-hannover.de/institute/pathologie/dgp). With the help of tissue arrays it becomes possible for the first time to distribute several tumours among a high number of participating pathologists, whereby almost identical tumour areas will be studied by all participants. The first and the final slide sectioned from a tissue-array block have a distance of less than a millimetre. Furthermore, potential hazards by tumour het-

erogeneity are neutralized by the high number of samples that are encompassed by a tissue array. Only suitable material pre-tested for reproducibility enters the trials. The tissue samples are selected by a panel of three independent and experienced pathologists. Tissue microarray slides with 20–30 tissue spots either negative or expressing ER at low, medium or high levels are distributed among the participants. Whereas the majority of laboratories (>80%) usually succeed in demonstrating ER positivity in the medium- and high-expressing tissue spots, less than 50% of participants obtain the correct results in tissue samples with low expression (von Wasielewski et al. 2002). Poor interlaboratory agreement usually is based on insufficient retrieval efficacy or suboptimal immunohistochemistry. Interobserver variability, which has been tested in the trials by reviewing all immunostains, is in most instances not responsible for aberrant evaluations (Mengel et al. 2002).

Participants fill out an accompanying questionnaire in order to gather information about antigen retrieval and detection methods. To enable improvements in those institutions which scored below average, the correlation between the methods applied and performance in the trial is communicated to all participants .

The trials to assess interlaboratory reliability of steroid hormone receptor evaluation are conducted each year. Recently, a comparable trial was unleashed for Her2/neu, which yielded satisfactory results with regard to immunohistochemistry as well as FISH. Only with the help of interlaboratory trials will it be possible to guarantee the reliable and standardized detection of target molecules in a non-centralized system of histopathological services.

Interlaboratory trials may be necessary, but they are not sufficient to assure reproducibility of immunohistochemistry and FISH. Additional controls have to be included and performed such as on-slide controls. The latter can be achieved with cell lines embedded in paraffin and sliced like ordinary tissue sections. Cell lines are preferable to tissue samples because a defined content of target can be attributed to individual cell lines. On-slide controls enable the correct evaluation of immunostains, even when slides are retrieved from the archive. Furthermore, clinicians and pathologists have to collaborate in order to ensure that adequate and rapid fixation of cancer tissue samples exploited for target analysis will take place according to standardized procedures.

In conclusion, pathology is facing a new challenge and will integrate more closely with therapy planning in oncology than ever before. In order to guide tumour therapy beyond typing and grading, new methods and standards of quality assurance have to be established in histopathology.

References

Cheson BD (2006) Monoclonal antibody therapy for B-cell malignancies. Semin Oncol 33 [Suppl 5]: S2–S14

Mengel M, von Wasielewski R, Wiese B, Rüdiger T, Müller-Hermelink HK, Kreipe H (2002) Interlaboratory and interobserver reproducibility of immunohistochemical assessment of the Ki67-labelling index in a large multi-centre trial. J Pathol 198:292–299

Mengel M, Kreipe H, von Wasielewski R (2003) Rapid and large-scale transition of new tumor biomarkers to clinical biopsy material by innovative tissue microarray systems. Appl Immunohistochem Mol Morphol 11:261–268

Rudiger T, Hofler H, Kreipe HH, Nizze H, Pfeifer U, Stein H, Dallenbach FE, Fischer HP, Mengel M, von Wasielewski R, Muller-Hermelink HK (2002) Quality assurance in immunohistochemistry: results of an interlaboratory trial involving 172 pathologists. Am J Surg Pathol 26:873–882

Rudiger T, Hofler H, Kreipe HH, Nizze H, Pfeifer U, Stein H, Dallenbach E, Fischer HP, Mengel M, Von Wasielewski R, Muller-Hermelink K, German Society for Pathology, Professional Association of German Pathologists (2003) Interlaboratory Trial 2000 „Immunohistochemistry“ of the German Society for Pathology and the Professional Association of German Pathologists (in German). Pathologe 24:70–78

Savage DG, Antman KH (2002) Imatinib mesylate—a new oral targeted therapy. N Engl J Med 346:683–693

Von Wasielewski R, Mengel M, Wiese B, Rudiger T, Muller-Hermelink HK, Kreipe H (2002) Tissue array technology for testing interlaboratory and interobserver reproducibility of immunohistochemical estrogen receptor analysis in a large multicenter trial. Am J Clin Pathol 118:675–682

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