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Review

Towards defining biomarkers indicating resistances to targeted therapies☆

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ABSTRACT

An impressive, but often short objective response was obtained in many tumor patients treated with different targeted therapies, but most of the patients develop resistances against these drugs. So far, a number of distinct mechanisms leading to intrinsic as well as acquired resistances have been identified in tumors of distinct origin. These can arise from genetic alterations, like mutations, truncations, and amplifications or due to deregulated expression of various proteins and signal transduction pathways, but also from cellular heterogeneity within tumors after an initial response. Therefore, biomarkers are urgently needed for cancer prognosis and personalized cancer medicine. The application of "ome"-based technologies including cancer (epi)genomics, next generation sequencing, cDNA microarrays and proteomics might led to the predictive or prognostic stratification of patients to categorize resistance mechanisms and to postulate combinations of treatment strategies. This review discusses the implementation of proteome-based analysis to identify markers of pathway (in)activation in tumors and the resistance mechanisms, which represent major clinical problems as a tool to optimize individually tailored therapies based on targeted drugs. This article is part of a Special Issue entitled: Biomarkers: A Proteomic Challenge.

1. Introduction

Protein kinases and their downstream signaling pathways control cell proliferation, apoptosis, motility, and the cellular metabolism and are often deregulated in cancers [1,2]. Kinase inhibitors like tyrosine kinase inhibitors (TKI) and inhibitors of the mammalian target of rapamycin (mTOR) are currently frequently used for the treatment of diverse solid and hematologic tumor types of distinct origin [3,4]. A total of 17 small-molecule kinase inhibitors have been approved for use as therapeutic agents for cancer [4], while even more are currently in the pipeline. In addition, four monoclonal antibodies (mAbs) acting on protein kinase targets have also been licensed for cancer therapy [4,5]. Biomarkers are of increasing importance for personalized medicine, with applications including diagnosis, prognosis, and selection of targeted drugs and drug combinations as well as individuals, who might benefit from this treatment. These drugs have been developed to target different signal kinases, such as HER2/neu, the vascular endothelial growth factor receptor (VEGFR), the epidermal growth factor receptor (EGFR), the Bcr-Abl kinase, the phosphatidylinositol 3 kinase (PI3K), mTOR, MEK/ERK and the janus kinase (JAK) within the JAK/STAT signaling pathway [6–12]. In addition, to the vast array of kinases, some non-kinases were inhibited resulting in a poorly detectable heterogeneous activity profile. These off target activities caused distinct and partially severe side effects, which can interfere with the efficacy of the treatment regime. Furthermore, patients often develop resistances to these drugs, and the prediction of a drug activity profile in order to avoid the treatment of patients with unforeseen and unfavorable risk profiles as well as the identification and early detection of resistance mechanisms are urgently required to optimize patient`s outcome and to select the appropriate therapeutic option. The rapid advances in "-omics" research including genomics, epigenomics, and proteomics led to the elucidation of the molecular signatures and functional pathways that underlie disease initiation and progression as well as to identification of molecular profiles that help the classification of tumor subtypes and determine their natural course, prognosis, and responsiveness to targeted therapies [13].

Abbreviations: 2 DE, 2-dimensional electrophoresis; ABPP, activity-based protein profiling; BFGF, basic fibroblast growth factor; CML, chronic myeloid leukemia; CRC, colorectal cancer; DIGE, differential in gel electrophoresis; EGF(R), epidermal growth factor (receptor); EMT, epithelial to mesenchymal transition; FGF 2, fibroblast growth factor-2; FT-ICR MS, Fourier transform ion cyclotron mass spectrometry; HGFR, hepatocyte growth factor receptor; ICAT, isotope-coded affinity tags; iTRAQ, isobaric tags for relative and absolute quantification; JAK, janus kinase; KRAB, Krueppl-associated box; LC, liquid chromatography; mAbs, monoclonal antibody; miRNA, micro-RNA; mTOR, mammalian target of rapamycin; NSCLC, non-small-cell lung cancer; PFS, progression free survival; PI3K, phosphatidylinositol 3 kinase; PTEN, phosphatase and tensin homologue; PTM, post-translational modifications; RCC, renal cell carcinoma; RTK, receptor tyrosine kinase; SELDI-TOF MS, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry; SILAC, stable isotypelabeling by amino acids in cell culture; TKI, tyrosine kinase inhibitor; VEGF(R), the vascular endothelial growth factor (receptor); WT, wild type; ZNF, zink finger

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2. Resistance mechanisms of targeted drugs: many causes one consequence

The major kinase inhibitors (VEGF, TKI and mTOR inhibitors) available for the treatment of different tumor types showed significant beneficial effects in terms of progression-free survival (PFS) and disease stabilization of patients [14]. Since responses are almost exclusively partial and tumors often develop progression during therapy within one year, strategies to prolong the effect of these drugs are of great importance. For example, all patients with metastatic renal cell carcinoma (RCC) eventually develop drug resistance and ultimately relapse [15]. Resistance to therapy may occur either directly linked to a primary unresponsiveness (intrinsic resistance), or it is acquired over time and related to various escape mechanisms developed by the tumor in response to therapy (extrinsic resistance) [16]. The unexplained pre-existing unresponsiveness of tumors to treatment resulting in a lack of clinical benefit could be mediated by inherited mutations of a kinase gene, which abolishes binding of inhibitory molecules [16]. In the case of VEGF inhibitors and TKIs, intrinsic resistances are due to the presence of pre-existing redundant pro-angiogenic factors promoting tumor angiogenesis, such as fibroblast growth factor-2 (FGF-2) and/or $CD11b^{+}Gr1^{+}$ myeloid cells [17–19]. Regarding mTOR-inhibitors, the proposed mechanisms of intrinsic resistance include the presence of redundant signaling pathways, the presence of K-RAS or B-RAF mutations, loss of phosphatase and tension homologue deleted on chromosome ten (PTEN), low cellular levels of p27 or 4EBP1, and overexpression of eIF4E [15,20]. However, mutations associated with a primary resistance or reduced sensitivity to pharmacologic intervention with targeted drugs are rare when compared to the acquired resistance [15,16]. The mechanisms involved in extrinsic resistance, also known as secondary, evasive, acquired, or adaptive resistance to anti-angiogenic therapies [15] encompass activation of the expression of alternative signaling molecules, which lead to an up-regulation of the existing pathways or to the recruitment of alternative factors responsible for tumor growth and/or revascularization. Both, TKI and VEGF inhibitors target components of the VEGF signaling pathway, and extrinsic resistance against these drugs ultimately results in evasion of the angiogenesis blockade [21]. However, the underlying mechanisms of resistance to these different targeted therapies are so far not fully understood and have been reported to involve in addition to restored angiogenesis, inhibition of angiostatic pathways and induction of angiogenesis independent processes, such as increased tumor invasiveness and epithelial to mesenchymal transition (EMT) [22–26]. Acquired resistances could also be mediated by distinct pharmaco-kinetic and pharmaco-dynamic barriers and include the induction of metabolism, solubility and protein-binding characteristics of the inhibitors. Furthermore, a selection of pre-existing populations harboring mutations or a small population of stem cells escapes inhibitor-mediated cytotoxicity. It has been shown in several hematologic tumors that quiescent stem cells are refractory to TKIs [3,27,28], since the bulk tumor could be repopulated resulting in survival and anti-proliferative advantages. The strong selective pressure for cells to acquire resistance through mutations in the kinase gene that abrogate drug binding leads to an enrichment of mutations of the 'gatekeeper residue' as a mechanism of secondary resistance. Under physiologic conditions kinase activity is unaffected and the accessibility of a hydrophobic pocket near the ATP binding site is prevented [3]. Since hydrophobic interactions in this site are essential for binding of the inhibitory molecules, these mutations could lead to inhibitory resistances in many cases [3]. Additionally, target amplification and up-regulation of alternative kinase pathways have been reported as non-mutation kinase inhibitory mechanisms leading to secondary resistances [4]. Examples thereof are the target amplification in the case of Bcr-Abl1 in chronic myelogeneous leukemia (CML) patients [29] and the up-regulation of the hepatocyte growth factor receptor (HGFR) in the acquisition of resistance to EGFR kinase inhibitors that has been observed in lung cancer [30]. Other mechanisms might be mediated by the removal of the oncogenic dependence of tumor cells bypassing the targeted pathways due the induction of compensatory signaling cascades for their survival or by additional kinetic alterations during prolonged treatment, which then leads to an insufficient inhibition by targeted drugs. The main mechanisms that are currently postulated to participate in the development of extrinsic resistance to mTOR, receptor tyrosine kinase (RTK) and growth factor inhibitors are summarized in Table 1 [15,31] and Fig. 1 [31]. Understanding the mechanisms of resistance may not only guide subsequent treatment selection, but may also provide insights into the optimal sequence of therapies.

Table 1
Primary target and potential mechanisms of resistance of targeted therapies.

Drug	Drugclass	Primary target	Diseases	Resistance mechanisms
Bevacizumab	mAb	VEGF-A	BrCa, CRC, NSCLC, OC, RCC	Activation of PDK1
Cetuximab	mAb	EGFR	CRC	*KRas*mutation, *BRaf*
Dasatinib	2nd TKI	Bcr-Abl, cKit, PDGFR	CML	TBD
Erlotinib	TKI	EGFR	NSCLC	*EGFR* or *HER2* exon 20 insertion, *KRas*mutation?, EGFR TK mutation, cMET amplification, PI3K/AKT activation
Everolimus	mTORinhibitor	mTOR, VEGF-A	RCC, kidney and heart transplantation	Up-regulation of IGF-1R, activation of AKT, Increase in ERK/MAPK pathway
Gefitinib	TKI	EGFR	NSCLC	*EGFR* or *HER2* exon 20 insertion, *KRas*mutation?, EGFR TK mutation, cMET amplification, PI3K/AKT activation
Imatinib	TKI	Bcr-Abl, cKit, PDGFR	CML, GIST	*Bcr-Abl* mutation, *cKit*, *PDGFR mutations*
Nilotinib	2nd TKI	Bcr-Abl, cKit, PDGFR	CML	TBD
Panitumumab	mAb	EGFR	CRC	*KRas*mutation, *BRaf*
Pazopanib	TKI	VRGFR, cKit, PDGFR	RCC	Recruitment of pericytes to maintain a portion of vessels permeable and functional and endothelial cells unaffected by anti-angiogenic therapies
Sorafenib	TKI	VEGFR, Braf, PDGFR	HCC, RCC	Recruitment of pro-angiongenic bone marrow-derived cells and monocytes
Sunitinib	TKI	VRGFR, cKit, PDGFR	GIST,RCC	*cKit*, *PDGFR* mutations (GIST), activation of alternative angiogenic signals
Temsirolimus	mTORinhibitor	mTORC1	RCC	Activation of PI3K/AKT, ERK/MAPK, PIM kinases and PDK1
Trastuzumab	mAb	HER2	BrCa	p95HER2, PTEN

2nd TKI: second generation TKI; BrCa: breast cancer; Braf: v-Raf murine sarcoma viral oncogene homolog B1; cKit: stem cell growth factor receptor Kit; cMET: macrophage-stimulating protein receptor; CML: chronic myeloid leukemia; CRC: colorectal cancer; EGF(R): epidermal growth factor receptor; GIST: gastrointestinal stromal tumors; HCC: hepatocellular carcinoma; HER2: Tyrosine kinase-type cell surface receptor HER2; IGF-1R: insulin-like growth factor-1 receptor; mAb: monoclonal antibody; mTOR (C1, C2): mammalian target of rapamycin (complex 1 or 2), NSCLC: non-small-cell lung cancer; OC: ovarian cancer; PDGF(R): platelet-derived growth factor (receptor); PDK1: pyruvate dehydrogenase kinase 1; PIM: serine/threonine-protein kinase PIM; PTEN: phosphatase and tensin homolog; RCC: renal cell carcinoma; TBD: to be determined; TK: tyrosine kinase; VEGF(R): vascular endothelial growth factor (receptor).

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