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Reversing Resistance to Targeted Therapy

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Summary

The development of molecular targeted anticancer drugs is rapidly changing cancer therapeutics. However, drug resistance to these novel agents remains a real clinical concern. Reports now indicate that resistance to many of these molecular targeted agents - including hormone therapies, trastuzumab, imatinib, and gefitinib - occurs via common resistance mechanisms. These include 1) inadequate target blockade due to sub-optimal drug delivery; 2) altered target expression at the DNA (gene amplification), mRNA or protein level; 3) an altered target such as a mutated kinase domain; 4) modified target regulating proteins (e.g. altered expression of co-activators and/or co-repressors for nuclear steroid hormone receptors); 5) signalling by alternative proteins (functional redundancy) or different signalling pathways. It is envisioned that the molecular evaluation of clinical anticancer drug resistance, which requires the detailed study of pharmacokinetics, pharmacogenetics and pharmacodynamics, will allow the development of rational reversal strategies and improved patient outcome.

Key words: **Targeted therapy, resistance.**

INTRODUCTION

Preferential cytotoxicity against malignant tissues remains tantamount to the Holy Grail in cancer therapeutics because this portends improved patient tolerance and quality of life and, importantly, the capacity to deliver combination therapy. Rationally designed and molecular target based anticancer agents are characterised by lower toxicity and wider therapeutic indices than traditional cytotoxic drugs. These agents may reverse or modulate chemotherapy resistance, enhance anti-tumour activity or maintain tumour regression. This article reviews potential mechanisms of resistance to targeted therapies and suggests strategies to overcome this resistance, thereby maximizing anti-tumour effect and clinical benefit.

SPECIFIC MECHANISMS OF RESISTANCE

Anti-androgen resistance

Hormone therapy remains the mainstay for the treatment of metastatic prostate cancer. Androgen deprivation therapy induces a remission in 80 to 90% of patients with advanced disease and results in a median progression-free survival of 12 to 33 months, at which time an androgen-independent phenotype usually emerges. This accounts for the median overall survival of 23 to 37 months from the initiation of androgen deprivation [1]. Various mechanisms of androgen resistance have been postulated. Inadequate LHRH and testicular androgen suppression or circulating low levels of adrenal androgens can result in failure to respond to hormone therapy. Improving or increasing LHRH analogue delivery

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can abrogate the former, while adrenal androgen synthesis can be inhibited by the administration of low doses of steroids or through the inhibition of key enzymes in the adrenal steroid biosynthesis pathways with agents such as ketoconazole or the CYP17 inhibitor abiraterone acetate [2]. Preclinical data indicate that most androgen-independent prostate cancers continue to express androgen receptor (AR) with AR signalling remaining intact, as demonstrated by the expression of prostate-specific antigen (PSA), and consistently increased levels of AR mRNA, despite castrate testosterone levels [3]. This could occur following binding of (low) levels of ligand to hypersensitive AR complexes or by androgen-independent activation of the AR. AR gene amplification resulting in increased receptor levels could also result in sensitisation of the cell to low ligand concentrations. Similarly, point mutations of the AR gene may alter the antagonistic effect on the AR receptor seen with steroid ligands, such as oestrogens, corticosteroids or even AR antagonists, to an agonistic one. Although these mutations appear to be uncommon in the clinic,[4] they could explain the anti-androgen withdrawal syndrome observed in approximately 10-15% of hormone refractory prostate cancer (HRPC) patients [5].

Hormone resistance in the majority of patients who do not have AR gene alterations but who retain active AR signalling may be explained by androgen-independent activation by growth factor signalling, which can directly activate the AR and its downstream pathways. This may occur through activation of the erb-B receptors due to their aberrant expression or the over-expression of their ligands [6]. In addition, over-expression of insulin growth factor (IGF)-I and fibroblast growth factor (FGF)-8b has been reported in HRPC; binding to their respective receptors can bypass the AR and activate downstream signalling [7,8]. Ligand-independent activation of the AR can also occur through the increased expression of co-activators (such as SRC-1) or decreased expression of co-repressors (such as NCoR), which can alter AR transcriptional activity [9]. Finally, alternative-signalling pathways involved in the regulation of apoptosis, such as the up-regulation of anti-apoptotic genes (e.g.Bcl-2 or the IAPs) could impart clinical androgen resistance. Strategies to reverse such resistance have been pursued in clinical trials utilising antisense technologies.

ANTI-OESTROGEN RESISTANCE

Intrinsic resistance or the development of acquired resistance to oestrogen manipulation remains a significant clinical problem in the treatment of oestrogen receptor (ER) positive breast cancer [10]. While up to 70% of patients with ER positive advanced disease initially respond to hormonal modulation, most tumours will eventually become resistant. While the most important factor in defining hormonal resistance is lack of ER expression, the mechanisms of resistance in ER positive tumours have not been fully elucidated and may be multifactorial.

The ER pathway is complex and involves multiple co-regulating factors linked to other signalling pathways and in particular, is regulated through its phosphorylation on two serine residues which can be modulated by the PI3K/Akt and the Ras/Raf/Erk pathways. The ER forms multiprotein complexes with co-regulatory proteins that possess either histone acetylase or histone deacetylase activity. These co-regulators are recruited in the process of ER ligand binding, increasing or blocking downstream effectors depending on the balance between co-activators and co-repressors. Increased ER expression can alter the response of ER binding to ligands such as oestrogens or anti-oestrogens. Changes in host endocrinology, drug pharmacokinetics, altered α:β ER ratios, ER mutations, perturbations in downstream growth factor signalling, and altered ER co-regulating factors have all been implicated in this resistance phenotype [10].

Reports have described a mutation in the hormone-binding domain of ER-α that is hypersensitive to low levels of oestrogen compared with wild-type ER-alpha in preclinical models [11]. This point mutation comprises an amino-acid substitution in the ER-α acetylation site [5] that alters the ability of the ER to bind to many co-regulatory factors and consequently, its transcriptional activity. However, this mechanism of resistance remains controversial. Up-regulation of receptor co-activators is another possible mechanism of oestrogen resistance. Reports indicate that increased expression of co-activators may stimulate growth in the absence of oestrogen [10]. In this environment, tamoxifen may exert an agonistic rather than an antagonist effect. Furthermore, cross-talk between erb-B receptor signalling and the ER has been associated with endocrine therapy-resistance [10,12]. The ER may be activated by phosphorylation in the absence of its ligand oestradiol by growth factor signalling (e.g. EGF and IGF-1) through both mitogen-activated protein kinase and Akt pathways. Preclinical and clinical data have already demonstrated that epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors may reverse resistance to tamoxifen, and clinical trials combining endocrine therapies and signal transduction inhibitors are ongoing [12]. Preliminary data from a phase II trial indicate that gefitinib is active in ER positive tamoxifen-resistant breast cancer patients [13,14].

Altered intracellular signalling, downstream of the receptor tyrosine kinases may also result in hormone resistance. This can be addressed by targeting downstream signal-transduction proteins that interact with the ER pathway; for example inhibiting Ras signalling by the farnesyltransferase inhibitors (FTIs) or

the molecular target of rapamycin (mTOR) by the rapamycin analogues [15,16]. Emerging clinical trial data indicate that these agents can reverse hormone resistance in advanced breast cancer.

TRASTUZUMAB (HERCEPTIN™) RESISTANCE

Many mechanisms may be involved in the development of resistance to trastuzumab. Redundant signalling mediated by the hetero- or homo-dimerization of other members of the erb-B family (EGFR, HER3, HER4) may activate downstream signalling despite HER-2 blockade by trastuzumab [17]. Trastuzumab resistance may be reversed by combining agents that target different members of the erb-B family (for example, trastuzumab with gefitinib or trastuzumab with erlotinib) or using agents that target more than one erb-B family member. Agents such as the human monoclonal antibody pertuzumab (Omnitarg/2C4) targeting the heterodimerization domain on HER2 or the pan-erbB small molecule inhibitors need to be evaluated in this setting. Trastuzumab resistance may also result from overexpression of other growth factor receptors or their ligands (such as the IGF-1R) or altered downstream proteins (eg. loss of PTEN) that modulate downstream signalling [18]. Targeting downstream signalling with small molecule inhibitors such as the mTOR inhibitors, Raf kinase inhibitors or FTIs may therefore reverse trastuzumab resistance. Finally, recent data suggest that some trastuzumab-resistant breast cancers express reduced $p27^{kip1}$ levels with increased cdk2/4 activity, suggesting a role for drugs that induce $p27^{kip1}$ protein expression or inhibit cdk2/4 in the treatment of these patients [19]. Small molecule cdk inhibitors are being evaluated in the clinic and may merit further evaluation in this setting.

GEFITINIB RESISTANCE

Gefinitib (Iressa™) is an EGFR tyrosine kinase inhibitor. Recent reports indicate that incomplete target blockade due to poor drug delivery may be a significant concern in the treatment of colorectal cancer [20]. Tumour biopsies from 28 colorectal cancer patients were collected before and after treatment with gefitinib (single agent gefitinib 250 mg vs 500 mg on an ECOG randomised phase II trial comparing the two doses given continuously daily). EGFR, p-EGFR (tyrosine residue at position 1068), Akt, p-Akt, MAPK, p-MAPK and Ki67 were characterized [20]. Only 3 tumours demonstrated a fall in p-EGFR after one week of gefitinib therapy while the remaining biopsies did not show p-EGFR inhibition indicating that gefitinib was not effectively inhibiting its target. No inhibition of downstream signalling or Ki67 expression was observed. In this study only 1 of 110 patients treated had an objective response. Other reports indicate that patients who develop an acneiform skin rash while on treatment with an EGFR-targeting drug are more likely to show clinical benefit. This was noted in studies of patients with non-small cell lung cancer (NSCLC) treated with erlotinib and patients with colorectal carcinoma treated with cetuximab (Erbitux™) [21]. The development of skin rash reflects target blockade, suggesting that patients with higher drug exposure are more likely to respond. In an attempt to maximise target blockade and minimise inter-patient pharmacodynamic variability, it has been proposed that dosing with these agents should be increased until a grade 1 or 2 (CTCAE) skin rash is observed.

Incomplete target blockade is only one of several potential resistance mechanisms to treatment with EGFR inhibitors. Increased expression of the target EGFR or its ligands due to gene amplification, altered RNA stability or altered recycling can all result in gefitinib resistance. Mutations of the EGFR kinase domain can also result in altered EGFR sensitivity to gefitinib. The EGFRvIII mutation that results in amino-acid terminal truncation and constitutive activation of the receptor has been reported to be less sensitive to small molecule TKIs than the wild-type receptor. Moreover, mutations in threonine 766 in the EGFR kinase domain confer resistance to quinazoline inhibitors [22]. This site appears to be a "hot-spot" for the development of resistance since mutation of the corresponding threonines in C-Abl, p38 and Src also confer resistance to their respective small molecule inhibitors [22]. Conversely, data also indicate that pre-treatment EGFR mutations can increase sensitivity to EGFR blockade by gefitinib [23]. Preliminary studies indicate that tumour samples from 9 NSCLC patients who responded to gefinitib were found to contain recurrent heterozygous single amino-acid mutations in the EGFR kinase domain (at exons 18, 19 and 21) in contrast to samples from the patients who did not respond that did not have any of these mutations. Mutated EGFRs were more sensitive to ligand stimulation and consequently, more sensitive to gefinitib.

Inhibition of EGFR phosphorylation can also be uncoupled from inhibition of downstream signalling by alterations in other signalling effectors, such as constitutively activating mutations of Ras, B-Raf and PI3 kinase or loss of expression of the Akt regulator PTEN [24] or activation of other tyrosine kinase or G-protein coupled receptors (eg other erbB receptors or the IGF-IR). Combinations of targeted inhibitors need further evaluation in the clinic.

IMATINIB RESISTANCE

Imatinib (Glivec™, Gliveec™), a small molecule inhibitor of BCR-ABL, C-kit and PDGFR-α, has had a major impact on the treatment of chronic myeloid leukaemia (CML) and gastrointestinal stromal

tumours (GIST). Several mechanisms of imatinib resistance have been reported. Incomplete target blockade due to inadequate drug delivery may be a reversible resistance mechanism. Reports indicate that in chronic phase, Philadelphia chromosome positive, CML higher doses of imatinib (600 or 800 mg daily) may overcome resistance to lower doses (400 mg per day) [25]. P-glycoprotein (mdr1) overexpression has also been reported to confer resistance to imatinib *in vitro* by reducing intracellular imatinib concentrations, although these findings remain controversial since other reports indicate that imatinib is a poor substrate for p-glycoprotein [26,27]. Other factors that decrease imatinib delivery to its target include high levels of alpha-1 acid glycoprotein (AGP), which can prevent intracellular penetration by binding imatinib [28].

Probably the most common mechanism of acquired imatinib resistance is alteration of the drug target [29-32]. Gene amplification of BCR-ABL and mutations of key amino acids in the ABL or c-Kit kinase domains have been reported. Increasing drug dose,[25] or concurrently administering Hsp-90 inhibitors that inhibit the expression of the mature target protein, may reverse the impact of BCR-ABL gene amplification [32]. Approximately 30 different point mutations, coding for distinct single amino-acid substitutions in the BCR-ABL kinase domain, have been isolated from relapsed CML patients resistant to imatinib. While it has been suggested that some of these mutations predate imatinib therapy, in most cases the selective pressure of imatinib treatment induces these mutations. Many of these mutations have been shown to alter the imatinib-binding region of the BCR/ABL kinase domain, thus reducing imatinib's binding affinity. These mutations occur either at sites that come into direct contact with imatinib (T315, F317, and F359), and presumably impair drug binding without significantly affecting the binding of ATP, or at residues located in distant regions implicated in the unique conformational change that the kinase domain must undergo to accommodate imatinib [29]. Crystallographic studies predict that most imatinib-resistant mutants should remain sensitive to inhibitors that bind ABL with less stringent conformational requirements than imatinib. Novel Abl kinase inhibitors are showing promising activity in preclinical studies and are in various stages of development [30]. Conversely, however, it is important to note that in GIST activating mutations of c-KIT frequently predate therapy with imatinib and are key driving mutations in this disease, predicting response and correlating with clinical outcome with imatinib[31].

BCR-ABL signalling may also be inhibited through the induction of BCR-ABL protein degradation using inhibitors of the molecular chaperone Hsp90 or the blockade of critical downstream signal transduction proteins [32,33]. Hsp90 inhibitors, such as 17-AAG, have potent BCR-ABL inhibitory activity and clinical trials with 17-AAG and imatinib in CML are underway. Preclinical studies also suggest that inhibition of farnesyltransferase, presumably through inhibition of Ras downstream signalling, may also reverse imatinib resistance [33].

BEVACUZIMAB (AVASTIN™) RESISTANCE

Angiogenesis inhibition has emerged as an important therapeutic strategy in the treatment of malignancy [34,35]. Tumour cells recruit endothelial cells by secreting pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). VEGF is overexpressed in many tumours and is associated with a worse clinical outcome. The aetiology of resistance to anti-angiogenesis agents has not yet been extensively explored. Nonetheless, similar mechanisms of resistance, as have been described for other targeted agents, are likely. Elevated pro-angiogenic ligand (eg VEGFs, FGFs) expression may contribute to antiangiogenesis drug resistance, and have already been reported to contribute to resistance to chemotherapy or endocrine therapy [35,36,37]. It is plausible that resistance to bevacuzimab, a humanised monoclonal antibody to VEGF that in combination with chemotherapy has resulted in a survival advantage in colorectal cancer, may result from increased expression of VEGF. This may be reversed by increasing bevacuzimab dosing. Rational combinations of angiogenesis inhibitors may optimise the antitumour effects of these therapeutics.

CONCLUSION: REVERSING RESISTANCE TO TARGETED DRUGS

Overall, a number of common resistance mechanisms have been reported for targeted therapeutics. These include 1) inadequate target blockade due to sub-optimal drug-delivery; 2) altered target expression at the DNA (gene amplification), mRNA or protein level; 3) an altered target such as a mutated kinase domain; 4) modified target regulating proteins; 5) signalling by alternative proteins (functional redundancy) or different signalling pathways. Several strategies for the reversal of resistance to targeted anticancer therapeutics can therefore be considered and may be widely applicable.

The first consideration to reversal of resistance involves the evaluation of whether maximal target blockade has been achieved. Increasing drug dosing can reverse resistance, although this may be limited by toxicity. Increasing drug delivery by decreasing intratumoral interstitial pressure through the inhibition of PDGF receptor signaling, may also reverse resistance [38]. Emerging clinical data suggest that differences in the therapeutic efficacy between monoclonal antibodies and small molecules targeting EGFR signaling in colorectal cancer may be due, at

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