Farnesyl transferase inhibitors – a novel therapy for breast cancer

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Abstract

Inhibitors of the enzyme farnesyl protein transferase prevent a key step in the post-translational processing of the Ras protein, and were developed initially as a therapeutic strategy to inhibit cell signalling in ras-transformed cells. As more has been learnt about the biological effects of farnesyl transferase inhibitors (FTIs) on cancer cells, it is clear that tumours without oncogenic ras mutations such as breast cancer may also be targets for FTI therapy. This article reviews the rationale for the development of FTIs, focussing on early preclinical data in breast cancer models together with preliminary results from the first phase II study of an FTI in advanced breast cancer.

Introduction

Proto-oncogenes which result in over-expression or aberrant function of their encoded protein represent an obvious target for development of novel therapies for breast cancer. The introduction into clinical practice of trastuzumab as a monoclonal antibody targeted against breast carcinomas which over-express the growth factor receptor HER-2/neu provides the first proof of principle for this approach (Slamon et al. 2001). However, an understanding of the signal transduction cascade downstream from growth factors and cell membrane receptor tyrosine kinases has revealed several other key proteins involved in malignant transformation, including the 21 kDa guanine nucleotide-binding proteins encoded by the ras proto-oncogene.

There are three ras proto-oncogenes (H-, N- and K-ras) which encode a total of four 21 kDa proteins (H-Ras, N-Ras, and K-Ras4A plus K-Ras4B which result from two alternatively spliced K-ras gene products) (Barbacid 1987). Processed Ras proteins localise to the inner plasma membrane, and play a critical role in intra-cellular signalling by functioning as a molecular switch which alternates between an inactive guanosine 5′-diphosphate (GDP)-bound form and an active guanosine 5′-triphosphate (GTP)-bound state (Fig. 1). As such, they transmit a variety of extracellular signals from the cell surface, including growth factors which activate cell surface receptors (e.g. EGFr, HER-2/neu, PDGFr), cytokines (e.g. interleukin (IL)-2, IL-3, GM-CSF) and hormones (e.g. insulin/insulin-like growth factor (IGF)) (McCormack 1994). Tyrosine kinase activation on either growth factor receptors (e.g. HER-2/neu or EGFr) or non-receptor proteins activated by cytokines (e.g. Src) allows an ‘adapter protein’ (Grb2) to recruit the Ras activator protein (SOS) to the plasma membrane, thus facilitating the exchange of GTP for GDP on the membrane-bound Ras protein.

Once in its GTP-bound form, Ras activates several downstream effector pathways which mediate either cell proliferation or other effects (Fig. 1). One such key event involves the recruitment of Raf-1 to the plasma membrane (Leevers et al. 1994). Activated Raf-1 phosphorylates the protein kinases MEK1 and MEK2 which activate the mitogen-activated protein kinase (MAPK) cascade, ultimately resulting in nuclear translocation of MAPK and transcription of downstream target genes involved in cell proliferation (Marshall 1996). Ras can also directly activate the serine-threonine protein kinase MEKK independently of Raf-1, a pathway which involves Jun N-terminal kinase (JNK) and leads to activation of the c-Jun transcription factor (Lange-Carter et al. 1993). In addition, Ras activates other downstream effector pathways including Rac and Rho which are small G proteins involved in the regulation of actin cytoskeleton and focal cell adhesions (Ridley & Hall 1992), and phosphatidylinositol 3′-kinase (PI3K), a family of lipid kinases which phosphorylate a series of second messenger phosphoinositides which contribute to cell survival through suppression of apoptosis (Kodaki et al. 1994, Kennedy et al. 1997). Thus, Ras activation has different cellular consequences which may depend on the upstream extracellular signals.
signalling and Ras isotype involved in any given cell type. In normal cells Ras signalling is tightly regulated, and in the absence of continued growth factor stimulation wild-type Ras rapidly reverts to its inactive state.

Role of Ras in human breast cancer

Point mutations in the ras gene are oncogenic or transforming because they result in a permanently active GTP-bound form of Ras. This was first demonstrated in bladder tumours when a sequence of tumour DNA was found to transform NIH 3T3 fibroblasts (Shih & Weinberg 1982). Subsequent analysis of human tumours identified that one of the ras genes, most frequently K-ras, contained point mutations especially in codons 12, 13 or 61. The encoded Ras protein contained an altered amino acid sequence which rendered the protein permanently activated due to the inability of the mutated form to interact with GAP/NF1, the enzymes normally responsible for rapid hydrolysis and inactivation of Ras-bound GTP to its GDP form (Lowry & Willumsen 1993). Mutant Ras proteins transform cells as they continuously activate the downstream effector pathways, including those involved in cell proliferation, in the absence of any upstream growth factor stimulation.

In the absence of any mutations in the ras gene itself, continuous activation of Ras protein may still occur in cells due to permanent upstream growth factor activation. Breast carcinomas are known to contain a very low frequency of Ras mutations (<2%) (Clark & Der 1995a), although aberrant function of the Ras signal transduction pathway is thought to be common in human breast cancer (Clark & Der 1995b). In transgenic mouse mammary tumours, receptor tyrosine kinase pathways result in activated Ras protein signalling (Norgaard et al. 1999), while homone-sensitive MCF-7 breast cancer cells have been shown to express high levels of Ras-related proteins (Clark et al. 1996). Likewise, in other cancers such as astrocytomas which do not contain oncogenic ras mutations, high levels of ligand-dependent and -independent growth factor receptors result in functional activation of the Ras pathway, and studies have confirmed high levels of activated Ras-GTP in human glioblastoma multiforme tumours (Guha et al. 1997). In human breast cancer, overexpression of p21 Ras protein has been associated with a more aggressive type of breast cancer which correlated with aneuploidy, loss of p53 function and HER-2/neu overexpression (Theillet et al. 1986, Rochlitz et al. 1989, Smith et al. 2000). Thus, a strategy of targeting Ras function in cancer need not limit itself to tumours with proven oncogenic Ras mutations.

Farnesyl transferase – a novel target

For the Ras protein to function in the signal transduction cascade, it must become physically associated with the inner surface of the plasma membrane which involves lipidation, or prenylation, of the protein. The first step involves farnesylation, which transfers a 15-carbon farnesyl isoprenoid group from farnesyl diphosphate (FDP) to form a thioether bond with the cysteine moiety in the C-terminal tetrapeptide sequence of the Ras protein (termed the CAAX motif, where C = cysteine, A = aliphatic amino acid, X = any amino acid). Subsequent biochemical processes involve proteolytic cleavage of the −AAX peptide, carboxymethylation of the new C-terminal farnesyl-cysteine
residue, and finally attachment of a fatty acid palmitate residue near the farnesylated cysteine. The processed farnesylated Ras protein becomes more hydrophobic and readily associates with the lipid bilayer of the plasma membrane, allowing it to cycle from its inactive GDP-bound state to the active GTP-bound state in response to upstream tyrosine kinase signalling (Fig. 1).

Farnesylation is the first and most critical step in the post-translational modification of Ras (Kato et al. 1992). The reaction is catalysed by the farnesyl transferase (FTPase) enzyme, an αβ heterodimer which mediates the S-alkylation of the cysteine residue. The substrate specificity for FTPase enzyme is determined by the amino acids which make up the CAAX motif, in particular the X residue (Moores et al. 1991). Thus, proteins in which X = methionine or serine are preferred substrates for FTPase, such as N-Ras (Cys-Val-Val-Met), K4A-Ras (Cys-Ile-Ile-Met), K4B-Ras (Cys-Val-Val-Met) and H-Ras (Cys-Val-Leu-Ser). Other non-Ras proteins may also be substrates for FTPase such as the nuclear lamins and protein tyrosine phosphatases (PTPs). Thus, as a critical step in the processing of Ras, it was anticipated that inhibition of farnesylation alone may be sufficient to abrogate the cell signalling and transforming function of Ras in cancer.

Farnesyl transferase inhibitors

The detailed understanding of the FTPase reaction and the substrate specificity for the enzyme led to the rational design of several different farnesyl transferase inhibitors (FTIs) (Fig. 2). Initial compounds came from an attempt to design substrate analogues that competed with farnesyl diphosphate (FDP), and although these inhibited Ras processing in H-Ras transformed NIH 3T3 fibroblasts at micromolar concentrations, they failed to demonstrate any anti-tumour activity in animal models (Leonard 1997, Rowinsky et al. 1999). A more successful approach was the rational design of several peptidomimetic compounds based on the C-terminal tetrapeptide CAAX sequence of Ras as the determinant for enzyme recognition (Reiss et al. 1990). Prodrugs such as L-744,832 inhibited the growth of >70% of tumour cell lines (Sepp-Lorenzino et al. 1995), and significantly inhibited the growth of spontaneous mammary tumours in H-ras transgenic mice without any systemic toxicity (Khol et al. 1995). This represented one of the first reports of FTI-induced tumour regressions in an in vivo model. Other prodrugs such as FTI-277 have been synthesised in which the central portion of the CAAX mimetic is replaced with a rigid spacer group (Qian et al. 1994). Others have combined the properties of an FDP analogue together with a peptidomimetic, such as the synthesis of the bisubstrate inhibitor BMS-186511, a drug which is three log-fold more selective for FTPase than GPTase and inhibits Ras signalling and transformed growth without any effects on normal cells (Manne et al. 1995).

An alternative approach has been the high throughput screening of natural products or compound libraries which led to the discovery of two unrelated compounds, Sch-66336 and R115777, both of which are orally active and have now entered clinical development (Fig. 2). Sch-66336 is a tricyclic compound which is a potent inhibitor of FTPase which inhibits the growth of several tumour cell lines as well as K-Ras transformed xenografts in vivo (Bishop et al. 1995).

In human xenograft studies, a wide variety of tumours including colon, bladder, lung, prostate and pancreas were growth inhibited in a dose-dependent manner, whilst prophylactic administration of Sch-66336 at a dose of 40 mg/kg per day delayed both tumour onset and growth (Liu et al. 1998). R115777 is an imidazole-containing heterocyclic compound, which inhibits the growth of several different tumour cell lines, including those which contain either the wild-type or mutant ras gene (End et al. 2001). In vivo experiments with oral twice daily dosing of R115777 (25–100 mg/kg per day) for 15 days showed dose-dependent growth inhibition of both human colon and pancreatic cancer xenografts (End et al. 2001).

Cytostatic and pro-apoptotic effects of FTIs

An important initial observation was that many of the cellular effects of FTIs appeared to be cytostatic rather than cytotoxic and that, following removal of FTI from the culture medium, cells reversed to their transformed phenotype (Prendergast et al. 1994). This implied that therapy might need to be continuous rather than intermittent, and has led to the investigation of several different dosing schedules in the phase I trials. Although FTIs exert a cytostatic effect in many cell culture and xenograft models due to either G1 or G2/M cell cycle arrest (Miquel et al. 1997, Sepp-Lorenzo & Rosen 1998), in transgenic models FTIs may induce significant tumour shrinkage through induction of apoptosis. In H-Ras-transformed fibroblasts this may relate to the inability of FTI-treated cells to adhere to the substratum, perhaps through an effect on RhoB function (Du et al. 1999a) and altered cell adhesion properties (Lebowitz et al. 1997). In K-Ras transformed cells, evidence exists that FTIs directly induce cyctochrome C release and caspase 3 activation independent of p53 (Suzuki et al. 1998).

In addition, data have suggested that the pro-apoptotic effects of FTIs in malignant, but not normal cells, may be enhanced if cell survival pathways are modulated at the same time. While activation of the PI3K (or AKT) pathway in Ras-transformed cells prevented FTI therapy with L-744,832 from inducing apoptosis, depriving cells of serum (which normally promotes cell survival through cytokine/insulin-mediated activation of AKT) allowed FTI therapy to
induce apoptosis in malignant Ras-transformed cells (Du et al. 1999b). These data shed light on the reason why, in some cell/tumour systems, FTIs appear cytostatic as other cell survival pathways may be activated or overexpressed.

We have treated hormone-sensitive oestrogen receptor positive (ER+ve) MCF-7 breast cancer xenografts established in athymic mice with increasing oral doses (25–200 mg/kg bid) of R115777 (Kelland et al. 2001). In addition to demonstrating dose-dependent inhibition of tumour growth, in the harvested tumours we showed both inhibition of cell proliferation and induction of apoptosis, together with expression of the cyclin-dependent kinase inhibitor p21 (Fig. 3). While the effect on growth appeared to be mainly cytostatic in this model, the biological changes in proliferation and apoptosis suggest that significant effects on tumour growth rate may be induced with this therapy.

**Clinical efficacy in breast cancer**

At least five FTIs have entered clinical development, and preliminary results from nine phase I studies have been published over the last 18 months (reviewed in Johnston 2001). Dose-limiting toxicities in these studies have included myelosuppression (neutropoenia and thrombocytopoenia), gastrointestinal toxicity, peripheral neuropathy and fatigue. However, schedule may be as important for toxicity as dose. For example, in the continuous oral dosing of Sch-66336 doses of 300–400 mg bid which were well tolerated on a 7- or 14-day schedule every 3–4 weeks (Hurwitz et al. 1999, Adeji et al. 2000a) became intolerable once 28 days of continuous therapy was reached (Eskens et al. 2001). Likewise for R115777, although doses of up to 1300 mg bid for 5 days every 2 weeks were achievable (Zujewski et al. 2000), chronic dosing was only possible at doses of 300 mg bid (Schellens et al. 2000), or with a 21-day schedule followed by a 7-day rest (Hudes et al. 1999). It remains to be established whether chronic dosing with any of these agents will result in other cumulative toxicities, or whether a 14- to 21-day schedule with a 1-week break will prove the most effective method of administration to optimise the therapeutic index. Confirmation of biological efficacy has been demonstrated in many of these studies by inhibition of prenylation of prelamin A in buccal mucosa cells with Sch-66336 (Adeji et al. 2000a), and inhibition of prenylation of marker proteins (e.g. HDJ2) in peripheral blood lymphocytes with R115777 (Holden et al. 2001).

We conducted the first phase II study of an FTI in breast cancer (Johnston et al. 2000). A total of 41 women with advanced breast cancer were treated with R115777 using a continuous oral 300 mg bid dose. All patients had either failed two lines of endocrine therapy or had ER–ve tumours, and 46% patients had received one line of chemotherapy for metastatic disease. Clinical activity was seen with four partial responses seen in sites of visceral and soft tissue disease (Fig. 4), together with stable disease for >6 months (range 6–12+ months) in an additional six patients. The drug was well tolerated, although myelosuppression was the most frequent drug-related toxicity. With chronic dosing after 12 weeks,
grade 3 sensory neuropathy was seen in six patients although nerve conduction studies were abnormal in only three of these patients. We have subsequently treated a further cohort of 35 patients with breast cancer using a schedule of R115777 (300 mg bid) for 3 weeks therapy followed by a week’s rest of therapy, a schedule which appears not to be associated with any significant neurotoxicity in the randomised phase III studies in colorectal and pancreatic cancer.

All primary tumours were assessed for mutations in either H-, N- or K-ras by PCR, but only one tumour was found to have a mutation at codon 12 in H-ras which is consistent with previously published data that breast carcinomas contain a low frequency of Ras mutations.

Figure 3 (A) Dose-dependent growth inhibition and (B) induction of apoptosis in MCF-7 human breast cancer xenografts treated for 6 days with the FTI R115777 at doses of 25, 50 or 100 mg/kg (means ± S.E.). (C) Detection of unprocessed nuclear membrane protein prelamin A correlated with the biological effects of FTI therapy on growth and induction of apoptosis.
Correlation of clinical response with tumour phenotype, in particular hormone (ER and PgR) and growth factor receptor expression (HER-2/neu and EGFr) was undertaken. There was no association of response with ER/PgR expression, but six of the nine patients who responded to R115777 had tumours which were HER-2/neu positive. Thus, in a tumour type in which wild-type ras may be driven by upstream growth factor overexpression, this phase II study has demonstrated that inhibition of signal transduction by FTIs can be effective in breast cancer.

Potential for combined therapy with FTIs in breast cancer

*In vitro* studies have suggested that FTIs may be combined with some conventional cytotoxic drugs, and that the anti-cancer effects may be additive or indeed synergistic in some systems. A series of combination experiments has been performed with Ras wild-type breast cancer cells, examining the effects of the L-744,832 in combination with doxorubicin, cisplatin, vinblastine, 5-fluorouracil and paclitaxel (Moasser et al. 1998). Whilst the effects on growth inhibition for FTI plus most of cytotoxic agents were merely additive, the combination of L-744,832 with paclitaxel was clearly synergistic. This effect was independent of the sequence of exposure, and appeared to be related to synergistic induction of G2/M cell cycle arrest. These initial findings have been supported by recent preclinical studies with both R115777 and Sch-66336 in combination with paclitaxel (Ranganathan et al. 1999, Skrzt et al. 1999, Shi et al. 2000). The recent finding that Sch-66336 prevents farnesylation of centromere binding proteins (Ashar et al. 2000) raises the possibility of a direct FTI effect on microtubule formation during the G2/M phase of the cell cycle which, in turn, may lead to enhanced sensitivity to the microtubule stabilising action of the taxanes.

There have been several published clinical phase I studies of FTIs in combination with chemotherapy agents used in breast cancer. L-778,123, Sch-66336 and BMS 214,662 have all been combined with paclitaxel (Khuri et al. 2000, Sharma et al. 2000, Bailey et al. 2001), while R115777 has been combined with docetaxel (Piccart et al. 2001), capcitabine (Holden et al. 2001) and trastuzumab (Schwartz et al. 2001). To date, toxicities appear predictable and manageable, again with evidence of activity in heavily pretreated patients, some of whom had been resistant to the given cytotoxic as a single agent. FTIs may also augment other treatment modalities and, in preclinical models, they have demonstrated radiation-sensitising properties in tumours in which H-Ras transformation has been thought to confer resistance to ionising radiation (Bernhard et al. 1996, 1998). Likewise, it will be important in subsequent studies to determine whether tumour shrinkage in response to FTI is correlated with dysregulation of the PI3K/AKT pathway in cancer cells. This has implications for breast cancer with the possibility of FTIs combined with endocrine therapies such as tamoxifen, which in part may operate through suppression of IGF/P13K signalling.

Target validation – role of biomarkers

Unlike the development of classical cytotoxic chemotherapy drugs, the selection of an optimal dose and schedule for FTIs in patients with advanced cancer is not solely dependent on finding the maximum tolerated dose in phase I studies using standard clinical toxicity criteria. It is much more likely that once sufficient drug has entered the malignant cell to switch off signal transduction, increasing the dose further will yield no further gain. Thus a threshold ‘biologically effective dose’ may exist for such agents. The measurement of surrogate biomarkers has been used to study the biological activity of FTIs in treated patients (Adeji et al. 2000b). For example, inhibition of protein prenylation in accessible tissues (i.e. peripheral blood lymphocytes or tumour) has been examined both in our breast cancer xenograft model (Kelland et al. 2000a)}
2001) and in serial blood samples from the phase II clinical trial (Johnston et al. 2000). Increases in the unprocessed forms of the nuclear intermediate filament protein lamin A can be detected following exposure to FTIs using a specific antibody directed against the unprocessed prelamin A protein. In our xenograft model, a threshold was found in detection of prelamin A which correlated with the dose (50 mg/kg) which induced the greatest growth delay and at which significant induction of apoptosis was seen (Fig. 3). This is a potentially useful biomarker for the clinical setting, and studies in FTI-treated tumour biopsies may be helpful in the selection of an optimal dose and schedule to inhibit protein farnesylation in vivo. Such ‘proof of principle’ studies may be important to determine the lowest biologically effective dose which allows prolonged therapy with FTIs.

Conclusion

Hope now exists that modern signal transduction inhibitors such as FTIs may provide greater tumour specificity and clinical effectiveness than existing systemic chemotherapy strategies. It remains to be seen how these therapies will be integrated into the current treatment strategies for breast cancer and, in particular, whether synergy with cytotoxic drugs such as the taxanes will be a more effective role than single agent therapy. However, FTIs represent one of the first small molecules that may be important to determine the lowest biologically effective dose which allows prolonged therapy with FTIs.

References

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