

Recent Advances in Understanding the Antineoplastic Mechanisms of Farnesyltransferase Inhibitors

Jingxuan Pan¹ and Sai-Ching Jim Yeung^{2,3}

Departments of ¹Leukemia, ²General Internal Medicine, Ambulatory Treatment, and Emergency Care, and ³Endocrine Neoplasia and Hormonal Disorders, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Abstract

Farnesyltransferase (FTase) inhibitors (FTI) have broad anti-neoplastic actions targeting both cancer cells and mesenchymal cells involved in tumor angiogenesis. The small GTPases H-Ras, Rheb, and RhoB and the centromere proteins CENP-E and CENP-F are relevant targets of farnesylation inhibition; however, their relative importance in the antineoplastic effect of FTIs may vary in different cell types at different stages of the cell cycle and at different stages in oncogenesis. Three recent studies argue that Ras-independent and perhaps even FTase-independent properties are important to the antineoplastic action of this class of drugs. In mice, genetic ablation of FTase does not abolish the oncogenic activity of Ras, limiting the original conception of FTIs as an effective means to target Ras in cancer cells. FTase may not be the sole molecular target of these agents, and one study has suggested that FTIs act by targeting geranylgeranyl transferase II. Lastly, we have obtained evidence that induction of reactive oxygen species and reactive oxygen species-mediated DNA damage by FTIs may be critical for their antineoplastic action as a class. Together, these findings may alter thinking about how to apply FTIs in the clinic. (Cancer Res 2005; 65(20): 9109-12)

Introduction

Farnesyltransferase (FTase) inhibitors (FTI) were designed to block the action of Ras oncoproteins, which depends on posttranslational modification by adding a farnesyl isoprenoid membrane anchor. While it is becoming clear that FTIs only partly target Ras, at best, exactly how these agents selectively target cancer cells has emerged as an important question. The results of early phase II/III studies suggest that the activity of FTIs as single agents is modest and generally lower than that obtained by standard cytotoxic drugs (1). Ongoing clinical studies are assessing the role of FTIs for early-stage disease or in combination with cytotoxic agents or with other molecular targeted therapies for advanced stage tumors. Further insights into the molecular mechanism of action of FTIs will help to better define their optimal use in combination with other chemotherapeutic agents in the treatment of cancer patients.

Thus far, the basic questions about the targets and the antitumor effects remain unclear. First of all, which farnesylated protein substrates are involved in the antineoplastic action of FTIs? Is FTase the only target of FTIs? If FTase is not the only target, then what other proteins are targets of FTIs that are responsible for the antitumor activity? In this minireview, we update information on these basic questions.

Requests for reprints: S. Jim Yeung, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 437, Houston, TX 77030. Phone: 713-745-4516; Fax: 713-745-3674; E-mail: syeung@mdanderson.org.

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Which farnesyltransferase substrates contribute to the antineoplastic action of farnesyltransferase inhibitors?

In the proteome, there are close to 300 proteins with a CAAX motif that are potentially farnesylated and over 20 proteins have been proven to be farnesylated (2). Among these, FTIs have been shown to inhibit the farnesylation of H-Ras, Rheb, RhoB, and centromere proteins (CENP-E and CENP-F) and the contributions of these proteins to the antineoplastic action of FTIs have been evidenced as follows.

Activating Ras mutations are present in ~30% of human cancers. The Ras family members are small GTPases occupying key positions in the cell surface growth receptor signal transduction pathways that regulate cell differentiation, proliferation, migration, and survival (3). FTIs were developed based on the idea of blocking malignant transformation by inhibiting the farnesylation of Ras, a posttranslational modification step required for association to the plasma membrane where the Ras proteins function. Blocking the oncogenic action of Ras by FTI can lead to up-regulation of Fas and down-regulation of vascular endothelial growth factor (4). In the presence of FTIs, K-Ras can serve as a substrate of geranylgeranyl transferase type I (GGTase I), such that K-Ras retains oncogenic function in cells. However, FTIs can still inhibit tumors with K-Ras mutations, demonstrating that the antineoplastic action of FTIs is to a large degree based on their effects on proteins other than Ras (Fig. 1).

Another mechanism by which FTIs may stop cancer growth is by interfering with bipolar spindle formation during transition from prophase to metaphase in mitosis (Fig. 1). Centromere proteins CENP-E and CENP-F are substrates for FTase but not GGTase I. FTIs do not affect the localization of these proteins to the kinetochores (5, 6). However, FTI-treated cells have ring-shaped chromosomes that are not aligned at the metaphase plate (5) and the association between CENP-E and the microtubules is altered (6). It is tempting to speculate whether this particular antineoplastic mechanism of FTIs contributes to the observed enhancement of antineoplastic activity of the combination of FTIs with antimicrotubule agents (7–10).

Inhibition of protein kinase B/Akt-mediated cell survival pathways may play a significant role in the antineoplastic action of FTIs. Lonafarnib inhibits Akt activity in head and neck squamous carcinoma cells (11) and decreases Akt expression in non-small-cell lung cancer cells (12). The mechanism by which FTIs regulate Akt expression and activity in cancer cells is unclear. Downstream from Akt in the signaling pathway, mammalian target of rapamycin occupies a key signal integration position. Akt can inhibit a complex consisting of the tuberous sclerosis gene products TSC1/TSC2 that turn off Rheb, which can activate mammalian target of rapamycin (Fig. 1). Rheb proteins are farnesylated (13). Activation of Rheb stimulates growth and cell cycle progression and FTIs can block the ability of Rheb to activate the mammalian target of rapamycin/S6 kinase signaling (13–15).

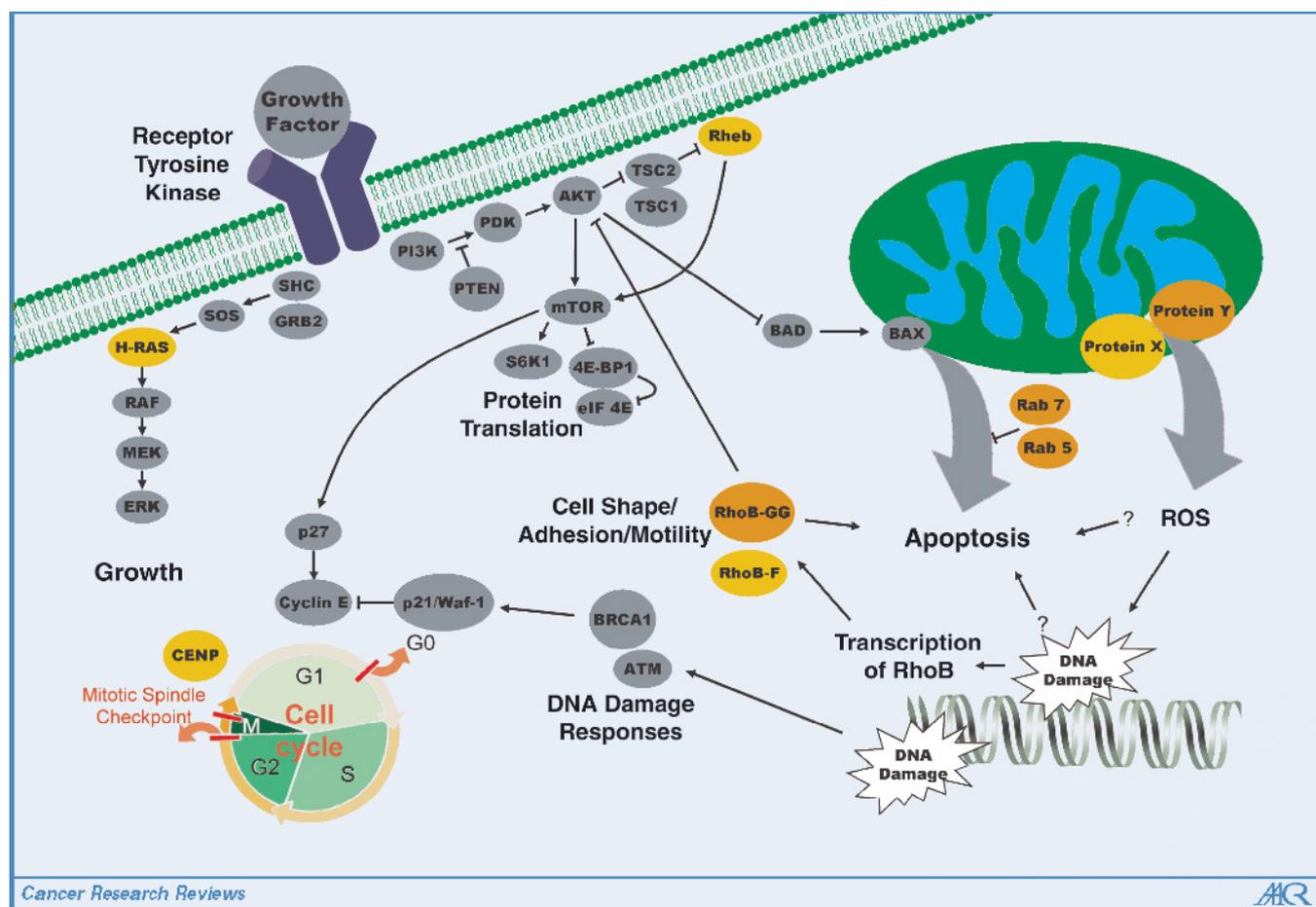


Figure 1. The mechanisms of antineoplastic actions of FTIs. The diagram depicts molecular targets of FTIs in cancer cells or mesenchymal cells involved in tumor angiogenesis. The farnesylated proteins (yellow) occupy key positions in pathways regulating proliferation, cell survival, cell cycling, and cytoskeletal regulation. Several geranylgeranylated proteins are colored gold. Increase in RhoB-GG is an important event in FTI-induced apoptosis. GGTase II, which prenylates the Rab proteins, may be a molecular target of certain FTIs in addition to FTase. Induction of ROS with ROS-mediated DNA damage subsequently links to induction of RhoB. The mechanism of ROS induction is unclear and may involve an unidentified farnesylated protein X or geranylgeranylated protein Y.

Rho proteins are involved in remodeling of actin cytoskeleton and in integrin-mediated cell adhesion (16). RhoA plays a key role in the regulation of actomyosin contractility. RhoB, which is localized primarily in endosomes, has been shown to regulate cytokine trafficking and cell survival whereas RhoC may be important in cell locomotion (17). Knockout mouse studies have implicated RhoB as an inhibitory regulator of integrin and growth factor signaling in oncogenesis (18). RhoB can be prenylated in cells by FTase or GGTase I to form farnesylated RhoB (RhoB-F) or geranylgeranylated RhoB (RhoB-GG), respectively. RhoB-GG is distributed in multivesicular late endosomes and RhoB-F at the plasma membrane (19). When farnesylation is blocked by FTIs, a shift of RhoB to RhoB-GG occurs. The antineoplastic action of FTIs may be mediated by a gain of RhoB-GG (20). Indeed, targeted deletion of RhoB abolishes the apoptotic response to FTI treatment, establishing an essential role for RhoB-GG in this response (21). cDNA microarray analysis revealed clusters of genes for mitogen-activated protein kinase signaling, cell cycle progression, and immune response as downstream proapoptotic mediators of RhoB (22). Suppression of cyclin B1 and Cdk1 (23) and inhibition of integrin and growth factor signals (21) by RhoB have been implicated in the apoptotic and antineoplastic actions of FTIs. Additionally, FTIs can reverse the RhoC-mediated phenotype of inflammatory breast cancer by an

increase in RhoB-GG (24), which may inhibit signaling by RhoC and other pro-oncogenic Rho proteins by sequestering common exchange factors or effectors such as protein kinase C-related kinase (25). Lastly, loss of RhoB-F by FTI may limit the survival of sprouting vascular endothelial cells that are present in growing tumors (26), suggesting a mechanism for the antiangiogenic effects of FTIs.

Is farnesyltransferase the only biochemical target for farnesyltransferase inhibitors?

In addition to FTase, eukaryotes have two additional prenyl transferases, GGTase I and Rab GGTase II. FTase and GGTase I are heterodimeric enzymes composed of a common α subunit and distinct β subunits with 30% identity to each other. In contrast, GGTase II has an α subunit with 27% identity to that of the CAAX prenyl transferases (FTase and GGTase I) plus additional domains and a β subunit with 29% identity to that of FTase (27). Nevertheless, the three enzymes have similar active sites (28, 29) and catalytic mechanisms (30).

Using a chemical genetics approach in *Caenorhabditis elegans*, Lackner et al. (31) identified GGTase II as an additional direct target of several proprietary FTIs from Bristol-Myers Squibb

(Princeton, NJ), which, unlike other FTIs, exhibit intrinsic cytotoxic activity. The abilities of these FTIs to induce apoptosis correlate with their potencies of inhibition of GGTase II. Additionally, GGTase II is overexpressed in ovarian, colon and lung cancers, and melanomas compared with normal tissue, and small interfering RNA inhibition of GGTase II in mammalian cancer cell lines causes apoptosis in a p53-independent manner (31).

Using a knockout mouse model in which the FTase β subunit gene was targeted for deletion, FTase was shown to be required for embryonic development but not for adult homeostasis. Additionally, FTase was found to be dispensable for the survival of cancer cells or tumor initiation but important for tumor progression (32). In agreement with these results, down-regulation of FTase β -subunit using small interfering RNA in cultured anaplastic thyroid cancer cells did not significantly affect cell survival (33). Although FTIs in clinical development showed selectivity toward FTase over GGTase I, the selectivity between FTase and GGTase II for these compounds is unclear. In addition, whether GGTase II represents another molecular target for FTIs other than the uniquely cytotoxic compounds developed at Bristol-Myers Squibb (31) remains to be clarified.

Direct induction of reactive oxygen species by farnesyltransferase inhibitors

Recently, our laboratory has shown that FTIs rapidly trigger the production of reactive oxygen species (ROS; refs. 33–35). Induction of ROS by FTIs may be a common phenomenon in malignant cells but not in normal cells. Among the consequences of ROS induction by FTIs was DNA damage (caspase-independent double-strand breaks, abasic sites, and generation of oxo-8-deoxyguanine; refs. 33, 35). Quenching ROS with *N*-acetyl-L-cysteine abrogated the FTI-induced DNA damage and antagonized the inhibitory effect of the FTI manumycin on accumulation of protein biomass.

The mechanism by which FTIs induce ROS is not yet clear. Theoretically, the intracellular level of ROS is the balance between ROS generation and elimination. A decrease in the expression of the antioxidant enzymes may increase ROS. However, we have found that the level and activity of superoxide dismutase is induced in ATC cancer cells in a delayed compensatory manner after treatment with the FTI manumycin.⁴ Therefore, FTI may elevate ROS primarily by increasing ROS generation. Mitochondria are the principal sites of ROS production. Manumycin induces an early and rapid mitochondrial morphology change (36). Whether this morphologic change is associated with ROS increase by FTIs remains to be investigated. In other experimental systems, the prenylated protein Rac1 is involved in ROS generation (37) and mutated nonprenylated Rac1 has markedly reduced ability to support superoxide generation (38). The mitochondrial complex II is at least partially involved in another system (39). In chronic myeloid leukemia cells, an increase in nitric oxide caused by FTIs may involve the induction of inducible nitric oxide synthase (34).

ROS generation is apparently an off-target activity of FTIs. First, the ability of FTIs to induce ROS did not seem to be a direct result of FTase inhibition because suppressing the expression of the FTase β subunit by 90% after small interfering RNA transfection was not sufficient to induce ROS. Second, although the amounts of ROS induced by FTIs were attenuated in FTase-silenced cells, this attenuation was small in magnitude when comparing with the 90% transcriptional suppression. Third, increase in ROS occurred 3 to 6 hours after treatment of FTIs, a time frame that is not expected to be sufficient to produce a significant effect on the steady-state level of farnesylated proteins except the ones with unusual rapid turnover rates. Taken together, ROS induction by FTIs seems to be largely independent of FTase inhibition.

DNA damage initiates various DNA damage response pathways. Several proteins are known to be involved in double-strand break repair [e.g., Brca1 functions in homologous recombination; DNA-dependent protein kinase and Ku70 function in nonhomologous end-joining]. In FTI-treated cells, phosphorylation and nuclear foci of Brca1 were observed, suggesting that FTI may affect key components of the homologous recombination repair pathway. We also found that ataxia telangiectasia-mutated and DNA-dependent protein kinase, but not ataxia-telangiectasia-mutated and Rad3-related, were involved in sensing the FTI-induced DNA damage. These DNA damage response proteins, particularly ataxia telangiectasia-mutated and Brca1, also provide links of FTI-induced DNA damage to cell cycle arrest and apoptosis.

FTI-induced ROS damage DNA and contribute to the antineoplastic effect of FTIs (33, 35). DNA damage responses include activation of DNA repair proteins and induction of RhoB (33). RhoB may sensitize cancer cells to DNA damage-induced apoptosis following genotoxic stress (18, 40). Quenching ROS did prevent induction of RhoB by the FTIs, suggesting that RhoB was induced by FTI-induced ROS-mediated DNA damage. Therefore, FTI-induced ROS, DNA damage, and DNA damage responses are linked to RhoB, linking these events in the complex mechanism of action of FTIs.

Clinical implications

FTIs were initially developed as a tactic to limit oncogenic Ras activity in cancer cells. However, it has become increasingly clear that the antineoplastic action of FTIs is based largely on Ras-independent and perhaps even FTase-independent mechanisms. Genetic studies in mice indicate that FTase is not essential for the activity of oncogenic Ras, underscoring the need to understand the role of other targets. In addition to molecular targets that are affected, ROS induction by FTIs may be critical to understanding the clinical activity of this class of compounds. Efforts to exploit this feature and to target cancers that rely on targets other than Ras may be important to leverage the fullest potential of FTIs in clinical application.

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⁴ Unpublished observations.

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