Protein farnesylation: Implications for normal physiology, malignant transformation, and cancer therapy

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Protein farnesylation is a lipid posttranslational modification required for the cancer-causing activity of proteins such as the GTPase Ras. Although farnesyltransferase inhibitors (FTIs) are in clinical trials, their mechanism of action and the role of protein farnesylation in normal physiology are ill understood. In this issue of *Cancer Cell*, two articles shed light on these important issues. Protein farnesylation was found to be essential for early embryogenesis, dispensable for adult homeostasis, and critical for progression but not initiation of tumorigenesis. Furthermore, Rab geranylgeranyltransferase was identified as a target for some FTIs. This minireview discusses the implications of these findings on normal physiology, malignant transformation, and cancer therapy.

Posttranslational modifications of proteins are pivotal to the regulation of their physiological function. While modifications involving phosphorylation and glycosylation have been well studied, lipid modification is an emerging field that is at present extensively investigated. In particular, prenylation, a lipid modification with intermediates in the cholesterol biosynthesis pathway, such as the 15-carbon farnesyl and the 20-carbon geranylgeranyl, is of great interest because many prenylated proteins are involved in signal transduction circuits whose dysfunction leads to cancer (Glomset et al., 1990; Sebti and Der, 2003; Zhang and Casey, 1996). There are 3 prenyltransferases: farnesyltransferase (FTase), geranylgeranyltransferase I, and geranylgeranyltransferase II (GGTase I and GGTase II). These 3 enzymes catalyze the covalent formation of a thioether bond between the prenyl group and the thiol group of cysteines at the carboxyl terminal of an estimated 300 proteins in the human proteome. FTase and GGTase I prenylate proteins which contain CAAX (C = cysteine, A = aliphatic, and X = any amino acid) at their carboxyl terminus, with FTase preferring proteins with X = Met, Ser, Ala, or Gln, and GGTase I preferring proteins with X = leu or Ile. FTase and GGTase I are heterodimers that share an α subunit and have homologous (30% amino acid identity) but distinct β subunits (Zhang and Casey, 1996). GGTase II (also known as RabGGTase) modifies proteins that usually end in CXC. RabGGTase is also a heterodimer, with its α subunit having 27% identity with the α subunit of FTase and GGTase I, and the β subunit showing 29% identity to the β subunit of FTase (Zhang and Casey, 1996).

Although a search of the Swiss-Prot database identified about 300 proteins which terminate with a CXXX and which are potentially prenylated (K. Zhu and S.M.S., unpublished data), only a fraction of these have been shown to actually be prenylated. Among documented farnesylated proteins are H-, K-, and N-Ras GDP/GTP binding GTPases, the nuclear lamins, and the kinetechores CENP-E and F. Geranylgeranylated proteins include the GTP/GDP binding GTPases, RhoA, RhoC, Rac1, cdc-42, and R-Ras (Kho et al., 2004; Reid et al., 2004). RhoB is found both farnesylated and geranylgeranylated in cells (Armstrong et al., 1995), whereas K-Ras becomes geranylgeranylated when FTase activity is blocked (Lerner et al., 1997; Rowell et al., 1997; Whyte et al., 1997). The fact that farnesylation and geranylgeranylation are required for the ability of Ras and Rho proteins to induce malignant transformation, invasion, and metastasis prompted many investigators to develop FTase inhibitors (FTIs) and GGTase I inhibitors (GGTIs) as novel anticancer drugs (Cox and Der, 1997; Downward, 2003; Gibbs and Oliff, 1997; Sebti and Der, 2003). To this end, many CAAX peptidomimetics as well as other FTIs and GGTIs have been developed that are highly potent and selective at inhibiting FTase and GGTase I in vitro and protein farnesylation and geranylgeranylation in intact cells. Furthermore, FTIs and GGTIs have been shown to inhibit proliferation and induce apoptosis in various biological systems. While GGTIs induce p21waf1, CDK inhibition, and Rb hypophosphorylation and arrest cells in the G1 phase of the cell division cycle (Sebti and Hamilton, 2000), FTIs in most cell types induce accumulation at prometaphase during mitosis (Ashar et al., 2000; Crespo et al., 2001). The antiproliferative and proapoptotic activity of FTIs appears to be related to their ability to inhibit the PI3K/Akt, mTOR, S6 kinase, and/or RheB pathways at least in some cancer cell lines (Castro et al., 2003; Jiang et al., 2000; Law et al., 2000; Liu and Prendergast, 2000; Tamanoi et al., 2001). While GGTIs are also potent inhibitors of this pathway, their ability to induce apoptosis may require suppression of survivin expression as well as inhibition of the PI3K/Akt pathway (Dan et al., 2004). Although these pathways appear to be involved in some human cancer cells, in others, the mechanism by which FTIs and GGTIs inhibit tumor growth and induce apoptosis is not known, and much more investigation is warranted to enhance our understanding of how these agents work. Finally, while many farnesylated proteins such as Ras, RhoB, RheB, CENP-E, and CENP-F have been proposed as targets for FTIs, direct proof that inhibition of the farnesylation of these proteins is involved in the mechanism of antitumor activity of FTIs is lacking (Prendergast, 2001; Sebti and Der, 2003)

The outstanding antitumor activity and lack of toxicity of FTIs in many animal models led to their quick approval for testing clinically. The results of these clinical trials are mixed, with positive outcomes in some settings such as in hematological diseases and breast cancer, but not in others such as highly metastatic advanced colon and pancreatic cancers (Adjei, 2001; Zhu et al., 2003). At present, it is not understood why

some patients respond to FTIs while others do not. While evaluating FTIs either as single agents or in combination in various clinical settings (i.e., early stages versus late stages of disease) may improve response rates, it is clear that we know little about how FTIs work, and that enhancing our knowledge of the mechanism by which FTIs inhibit proliferation and induce apoptosis will result in great benefit to cancer patients (Zhu et al., 2003). Among the most important questions that remain unanswered concerning the mechanism of FTI mode of action are:

(1) Is the housekeeping enzyme FTase the only biochemical target for FTIs? Are there other targets to FTIs that contribute to their antitumor activity? If so, are these important to tumor survival only in those patients that respond to FTIs?

(2) If FTase is the biochemical target for FTIs, then which FTase substrates are pivotal to malignant transformation (the inhibition of farnesylation of which results in thwarting aberrant signal transduction circuits that are important for tumor survival)? Are these farnesylated proteins only implicated in tumor survival of those patients who respond to FTIs?

(3) How important is protein farnesylation in normal versus tumor cells, and can we exploit the differences (if any) to avoid toxicity and undesirable effects? If protein farnesylation is critical to tumorigenesis, at what stage (initiation, progression, and/or maintenance) are tumors addicted to protein farnesylation?

Answering these questions will have a major impact on enhancing our ability to use FTIs in the prevention and/or therapy of cancer. In this issue of *Cancer Cell*, two reports help us get closer to reaching this important goal. The first, by Mijimolle et al. (2005), addresses in an elegant fashion the importance of protein farnesylation in embryogenesis, postnatal development, adult homeostasis, and tumorigenesis in mice. While much of the work on the importance of protein farnesylation has relied on chemical biology approaches using FTIs, this study used a genetic approach by generating constitutive and conditional knockout mice for the β subunit of FTase. The results clearly demonstrate that the FTase β gene is essential for early embryonic proliferation, but dispensable for postnatal development and adult homeostasis. Furthermore, this gene was not found to be required for initiation of tumorigenesis in a K-Ras-dependent lung tumorigenesis model or in a skin carcinogenesis model. Importantly, however, the study demonstrated that the FTase β gene is critical during tumor progression in the same chemical skin carcinogenesis model. These key observations enhance our understanding of the role of protein farnesylation in normal physiology, tumor development, and cancer therapy and prevention. First, the fact that mating FTase β (–/+) mice did not yield homozygous embryos at embryonic stages E11.5 or older due to decreased proliferation and increased apoptosis at E7.5, coupled with the demonstration that FTase β (–/–) MEFs have reduced proliferative activity and motility, confirms in a more direct way the earlier observation that farnesylated proteins play key roles in the cell division cycle, cytoskeleton organization, migration, and survival (Sebti and Der, 2003). Second, except for delayed wound healing and defects in erythroid cells, FTase β (–/–) conditional knockout mice up to 18 months of age did not show behavioral, anatomical, or pathological defects, suggesting that protein farnesylation is dispensable for postnatal development and adult homeostasis. This is somewhat surprising, and leads one to wonder how an organism can survive with potentially several hundred defective farnesylated proteins with various important physiological functions. Possible ways by which the organism could compensate for the loss of protein farnesylation include the fact that some farnesylated proteins may become geranylgeranylated by GGTase I (e.g., K-Ras), that alternative mechanisms to localize to cellular membranes could exist (e.g., palmitoylation), and that alternative pathways, not requiring farnesylated proteins, may accomplish the same physiological functions. Regardless of the mechanism of compensation, the fact that the lack of FTase activity has little affect on adult homeostasis in mice gives further support for the safe use of FTIs in humans, and is consistent with clinical trial results where FTIs were found to be well tolerated in most patients at doses where clinical activity is observed (Adjei, 2001). Furthermore, the maturation defects in erythroid cells of adult FTase β (–/–) mice are also consistent with some of the side effects observed in FTI clinical trials (Adjei, 2001). The third important observation made by Mijimolle et al. is that protein farnesylation is not required for lung tumorigenesis in a K-Ras-dependent mouse model. This is not surprising, considering the large body of evidence demonstrating that K-Ras is geranylgeranylated by GGTase I when FTase is inhibited (Lerner et al., 1997; Rowell et al., 1997; Whyte et al., 1997). If the observation that K-Ras can induce lung tumors in the absence of protein farnesylation in mice can be extrapolated to humans, then lung cancer patients with tumors where K-Ras drives oncogenesis would most likely be resistant to FTIs. This reasoning is in agreement with phase II clinical trials in lung cancer patients where little clinical activity was seen (Adjei, 2001). The fourth conclusion that can be drawn from this study is that protein farnesylation is not required for initiation but is very important for tumor progression during chemical (DMBA + TPA) skin carcinogenesis. This suggests that farnesylated proteins are not required for the initial "hit" that converts cells from normal to early malignant transformation, but are critical for tumor progression and maintenance, since removal of FTase β after DMBA/TPA initiation significantly reduced the number as well as size of skin papillomas. The important finding that tumor progression is hampered by the lack of protein farnesylation provides proof-of-principle for the use of FTIs as chemopreventive agents. Indeed, FTIs have been shown to be effective in mouse lung cancer chemoprevention models (Lantry et al., 2000; Zhang et al., 2003). One possible use for FTIs in prevention trials is after surgical removal of tumors of lung and breast cancer patients, where the incidence of recurrence is significant.

An important question that was not fully addressed by this study and that requires further investigation is whether protein farnesylation is required for tumors that are driven by H-Ras. This is an important question, because unlike K-Ras, H-Ras is exclusively farnesylated and is not alternatively geranylgeranylated when FTase is absent (Lerner et al., 1997; Rowell et al., 1997; Whyte et al., 1997). Although H-Ras is reproducibly activated in the DMBA/TPA skin carcinogenesis mouse model, only two-thirds of the papillomas contain mutant H-Ras (Balmain and Pragnell, 1983), indicating that mutant H-Ras is not the only contributor to the DMBA/TPA-induced tumors. An alternative model that could more directly address the importance of protein farnesylation in H-Ras driven oncogenesis is one where the FTase β (–/–) mice are crossed with the MMTV-H-Ras transgenic mice where mammary tumor formation is driven by mutant H-Ras (Sun et al., 2003). Finally, Mijimolle et al. made an intriguing observation that warrants further investigation. They found that, in FTase β (–/–) MEFs, H-Ras is not farnesylated but is associated with 100,000 × g pellets, suggesting that it is still membrane-bound. This is an important finding that must be

confirmed by showing by immunohistochemistry in whole cells that H-Ras is still localized to the inner leaflet of the plasma membrane. Furthermore, the mechanism by which this association occurs is of great interest. What is puzzling about this finding is that the C186S H-Ras mutant, which is not prenylated, and H-Ras from FTI-treated cells are both cytosolic and not membrane-bound (Lerner et al., 1995; Willumsen et al., 1984a; Willumsen et al., 1984b).

The second article deals with the important issue of whether targets other than FTase can contribute to the antiproliferative and proapoptotic effects of some FTIs. Using a chemical genetics approach, in the nematode c-elegans as a model, Lackner et al. (2005) identified inhibition of RabGGTase as a potential mechanism by which some FTIs can induce apoptosis. The authors demonstrated that certain FTIs from Bristol-Myers Squibb that are potent inducers of apoptosis directly inhibit the enzymatic activity of RabGGTase with potencies that correlate well with their p53-independent proapoptotic activity. The proapoptotic activity of FTIs was mimicked by knockdown of RabGGTase as well as some of its substrates such as Rab5 and Rab7 that are involved in endosomal trafficking. Finally, the authors also found that overexpression of both the α and β subunits of RabGGTase is prevalent in human tumor tissues, providing further proof-of-concept for RabGGTase as a novel target for cancer drug discovery.

The findings of this second article have major implications for identifying novel targets for cancer therapy. Furthermore, if these findings can be extended to other FTIs, particularly to those presently in clinical trials, this could lead to a better understanding and interpretation of FTI clinical results. The first major implication for cancer therapy is the identification of a novel p53-independent pathway for inducing apoptosis in cancer cells. The authors provide strong evidence that demonstrates that inhibition of RabGGTase, which results in blocking the posttranslational modification of Rab proteins, leads to programmed cell death, suggesting that interfering with endosomal trafficking pathways is a novel approach to cancer chemotherapy. The authors' elegant knockdown experiments of various components of these pathways (i.e., Rab5 and Rab7), coupled with recent reports of the involvement of endosomal Rab GTPases in survival and proliferation of cancer cells, give further validation for this concept. Although the GTPases Rab5 and Rab7 could be considered as targets for developing novel proapoptotic anticancer drugs, RabGGTase is clearly a better target for structure-based rational drug design, since its structure and biochemistry have been studied (Shen and Seabra, 1996; Thoma et al., 2001a; Thoma et al., 2001b; Zhang et al., 2000). Furthermore, because of active site similarities with FTase and GGTase I (Strickland et al., 1998; Zhang et al., 2000), one can build on the extensive experience with the design of FTase and GGTase I inhibitor to design selective RabGGTase inhibitors (Reid and Beese, 2004). One drawback of this approach, however, could be blood clotting problems and retinal degeneration associated with inhibition of RabGGTase (Seabra et al., 2002).

The other major implication of the findings by Lackner et al. is that certain FTIs can potently inhibit RabGGTase, suggesting that these compounds have targets other than FTase that contribute to their antitumor activity, particularly their proapoptotic activity. This is important since it demonstrates for the first time that, though FTIs were designed/identified to inhibit selectively FTase, they can recognize other targets; in this case, a related prenyltransferase family member, RabGGTase. Furthermore, at present, it is not known why some tumor cells (in culture in vitro and in patients in vivo) are sensitive, whereas others are not. The discovery that certain FTIs can potently inhibit RabGGTase opens a new avenue to address this important question. It would, therefore, be very important to determine whether there is a correlation between the levels of expression of RabGGTase α and β subunits and/or inhibition of RabGGTase and clinical activities/toxicities seen in clinical trials. If this turns out to be the case, then one could propose a mechanism of selection for patients that would most likely respond to certain FTIs.

Although the findings of Lackner et al. are important, their interpretations and implications must be accompanied by a cautionary note. Clearly, further investigations are warranted to establish how universal the findings are and whether they can be extended to other unrelated FTIs. The important work described in this article, as pointed out by the authors, was prompted by the uniqueness of certain Bristol-Myers Sqibb FTIs, particularly BMS-1, as strong inducers of apoptosis. Although several FTIs have been evaluated by the authors, it is not clear from this article whether this unique character of certain FTIs can be extended to other FTIs, particularly those that have been extensively tested clinically. For example, if the clinical responses and toxicities that have been determined to date in the clinic are seen with FTIs that do not inhibit RabGGTase, then biochemical correlative work with this enzyme would not enhance our interpretations of clinical trial results as proposed above. Therefore, it is pivotal that a thorough and systematic evaluation of all known FTIs for their ability to inhibit RabGGTase be performed prior to committing to these correlations.

In summary, the articles by Mijimolle et al. and Lackner et al. describe highly elegant studies that enhance our understanding of the role of protein prenylation in embryogenesis, postnatal development, adult homeostasis, tumorigenesis, and cancer therapy and prevention. They have greatly advanced the field and will have consequences on future directions for mechanistic studies as well as investigations in clinic. The following are some important questions that are prompted by these studies and that warrant further investigations. What are targets other than FTase that contribute to FTIs' mode of action? Is RabGGTase a universal target for FTIs, particularly those that are in clinical trials? Can RabGGTase be a safe target for cancer therapy? Is protein farnesylation important for H-Ras-dependent oncogenesis? What farnesylated proteins are required for tumor progression and maintenance? Can FTIs be used as chemopreventive agents?

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