Farnesyl transferase inhibitors: the successes and surprises of a new class of potential cancer chemotherapeutics

Proteins that transmit abnormal growth signals offer enticing points of intervention for the treatment of cancer. The discovery that isoprenoid attachment is required for the aberrant biological activity of oncogenic Ras proteins has provided just such a target.

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For more than two decades the family of Ras proteins has been under intense study because of their central role in cell growth and their intimate association with an estimated 30 % of all human cancers [1]. Ras proteins must be localized to the plasma membrane in order to transform cells, and covalently attached lipids are the major impetus for this membrane interaction (Fig. 1) [2]. This discovery has led to the idea that preventing attachment of these lipids might disrupt the aberrant growth signals of oncogenic Ras proteins [3] and has propelled development of the first drugs designed to inhibit a lipid transferase. Several such compounds are now ready for rigorous testing in animal models of tumor growth.

Four central questions need to be answered before such inhibitors become useful. Will the inhibition of lipid transfer completely or partially eliminate the activity of oncogenic Ras? If the normal route of lipid transfer to Ras is blocked, can other mechanisms be used to activate Ras instead? Can other protein(s) or signaling pathways unaffected by inhibition of lipid transfer function as a surrogate for Ras? And, finally, will the activity of other proteins that share the same lipid transfer pathway as Ras be compromised and lead to unmanageable toxicity?

A primer on protein prenylation

Isoprenoids have been found only on three classes of proteins, defined by the presence of specific carboxy-terminal signals for three distinct prenyl transferase enzymes. Two classes of prenylated proteins have what are called CaaX motifs, composed of a cysteine followed by two (usually but not always) aliphatic residues and a carboxy-terminal 'X' residue. The amino acid in the 'X' position is important in determining which prenyl transferase will recognize the protein substrate [2]. Proteins with X = Leu (CaaL) are recognized by geranylgeranyl transferase I (GGTase I) and modified with the C_{20} isoprenoid geranylgeranyl. CaaL proteins are much more numerous than those in the other CaaX class, in which the X is Met, Ser, Ala, or Gln. Ras proteins are in this latter group, and are modified with a C_{15} isoprenoid by a farnesyl transferase (FTase). In general, proteins isolated from cells are modified by a single size of isoprenoid, rather than a mixture of C_{15} and C_{20} lipids (but see two important exceptions below). It is not yet

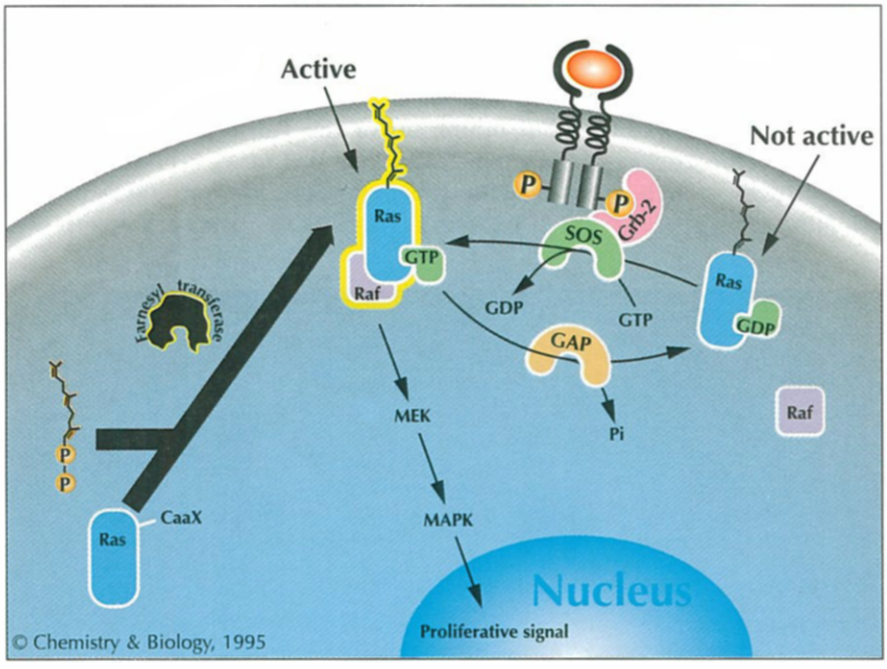


Fig 1. Ras and Ras farnesylation in cell proliferation. The binding of a growth factor to a receptor tyrosine kinase leads to conversion of inactive Ras (GDP) to active Ras (GTP). The protein kinase Raf then interacts with the GTP form of Ras and is thus recruited to the membrane. The now active Raf initiates a phosphorylation cascade that transmits a proliferative signal to the nucleus. The activity of Ras is dependent on its membrane localization, which is in turn dependent on the attachment of a C_{15} farnesyl group by FTase to the CaaX motif (where a is an aliphatic residue and X is Met, Ser, Ala or Gln) at the carboxyl terminus of Ras.

known whether inhibitors of FTase can cause some proteins that are normally modified with C_{15} lipid to switch to C_{20} modification. The third class of prenylated proteins have a carboxy-terminal Cys-X-Cys or X-Cys-Cys motif and are modified by a distinct geranylgeranyl transferase, GGTase II. Current FTase inhibitors cause no detectable changes in the prenylation of this third class of proteins.

Design of farnesyl transferase inhibitors

Routes to inhibitors of Ras farnesylation are apparent from an examination of the substrate specificities of the enzyme. One can design analogs either of the lipid, or of the peptide sequence to which the lipid is transferred. Such compounds must be stable, and readily cross the cell membrane to gain access to the cytosolic transferase.

Two examples of farnesyl diphosphate analogs are shown in Table 1. Both α-hydroxyfarnesyl phosphonic acid [4] (HFP) and a β-carboxy phosphonic acid derivative [5] (FCP) are potent inhibitors of FTase, showing >1000-fold selectivity over GGTase I or squalene synthetase *in vitro*. Esterification of the carboxylate group of FCP improves cell penetration. The FCP prodrug inhibits the growth of H-Ras-transformed NIH 3T3 cells at 100 μM, but also inhibits protein geranylgeranylation [5].

Non-substrate peptide inhibitors based on the CaaM motif, which is recognized preferentially by FTase, prove more potent and selective than the lipid analogs. Modification of the backbone of the CaaM tetrapeptide with reduced amide, olefin, or ether linkages gives inhibitors (such as B581 and L-739,750; see Table 1) that are less readily degraded *in vivo* than the original peptide [6,7]. Synthesis of a prodrug of L-739,750 in which the terminal carboxylate is masked by esterification gives a compound that inhibits Ras farnesylation and membrane binding in cells at low concentrations (0.1–1 μM) [8]. Such compounds also reduce colony formation of H-Ras-transformed cells in soft agar at 1 μM and block growth of H-Ras tumor xenografts in nude mice [9]. The growth obstruction of these compounds appears to result from inhibition of the Ras pathway, as the growth of cells transformed by Raf or mos, proteins which function downstream of Ras, is not affected.

The small tetrapeptide recognition sequence of FTase provides a guide from which small peptidomimetics can be produced. Replacement of the two central hydrophobic residues of the CaaX motif with aminobenzoic acid [10] or aminobenzodiazepine scaffolds [11] yields inhibitors with nanomolar affinity and >50-fold specificity for FTase (for example, FTI-276 and BZA-2B; see Table 1). An ester prodrug of BZA-2B slows the growth of H-Ras-transformed cells, and the ester prodrug of FTI-276 completely blocks farnesylation of H-Ras in transformed NIH 3T3 cells at 1 μM, and reduces the growth of Ras tumors in nude mice at a dose of 50 mg kg^{-1} per day [12] (see below). Future peptidomimetic design efforts will benefit from recent NMR spectroscopy data showing that the FTase-bound conformation of the peptide CVWM most closely resembles a type III β-turn (Fig. 2) [13]. A completely non-peptidic, tricyclic inhibitor (SCH 44342) [14], and bisubstrate inhibitors in which an active site model is used as a design template and an acyl-phosphinic acid used to link farnesol and the tripeptide, Val-Val-Met (BMS 185878) [15], have shown impressive effectiveness in intact cells.

The surprising successes of FTase inhibitors

The incentive to pursue these inhibitors has grown stronger with recent reports that tumors in whole animals respond dramatically to inhibitor treatment. The prodrugs of L-739,750 and FTI-276 have both been shown to block the establishment or slow the growth of tumors derived from H-Ras-transformed cells transplanted into nude mice [9,12]. The prodrugs fail to affect tumor cells that are transformed with Raf, the protein immediately downstream of Ras in the receptor tyrosine kinase signaling pathway, demonstrating that the effects are due to inhibition of Ras farnesylation. These inhibitors were similarly successful in preventing tumor growth in nude mice using malignant human lung cells [12] or human pancreatic adenocarcinoma cells [9] harboring mutations in the *myc* or *p53* genes in addition to K-Ras. The propyl ester of L-739,750 has also been shown to perform exceedingly well in reducing established mammary and salivary tumors in transgenic mice that overexpress the v-H-Ras oncogene [16], causing complete regression of 100 % of small tumors (seven of seven) and partial regression in 60 % of large tumors (three of five) when

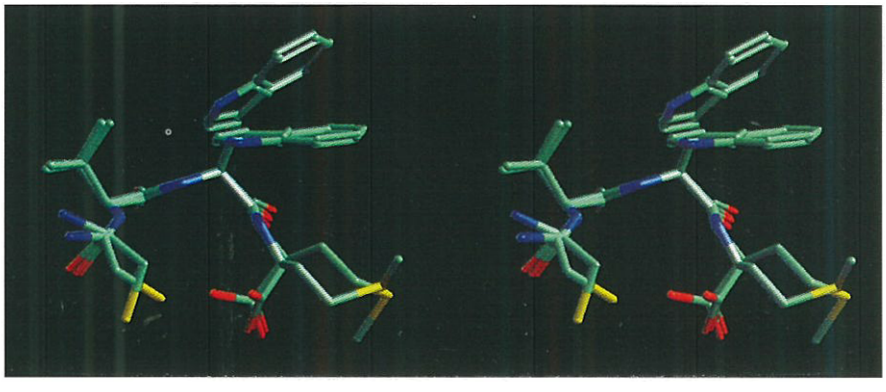
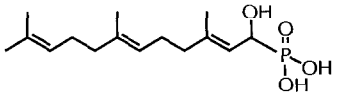
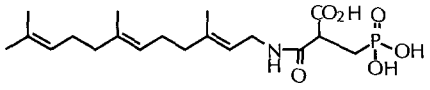
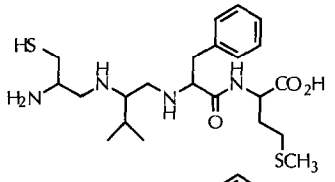
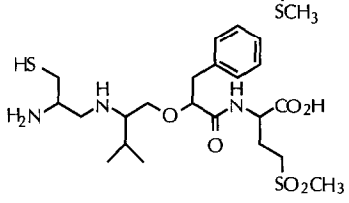
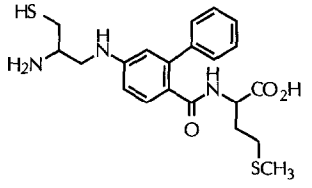
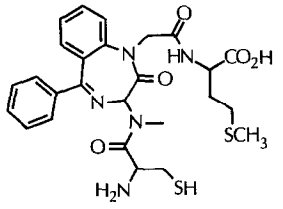
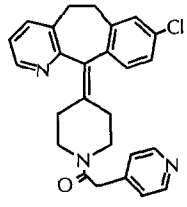
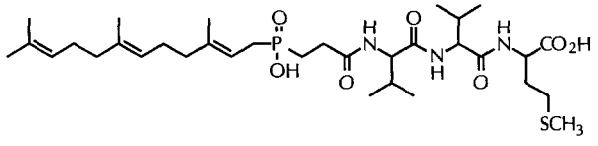


Fig. 2. Stereo view of the low-energy conformation of the peptide CVWM bound to FTase. The structure, as determined by NMR spectroscopy, most closely resembles a type III β-turn. Reprinted with permission from [13].

Table 1. Inhibitors of farnesyltransferase.

Structure	Compound	IC_{50} (nM)		ref.
		FTase	GGTase	
	HFP	30	35,800	[4]
	FCP	83	26,000	[5]
	B581	21	790	[6]
	L739,750	1.8	3,000	[9]
	FTI-276	0.5	50	[19]
	BZA-2B	0.8	35	[11]
	SCH44342	280	>12,000	[14]
	BMS-185878	6	21	[15]

administered to the mice at 40 mg kg^{-1} per day (Fig. 3). This response rate was comparable to that produced by a more traditional chemotherapeutic, doxyrubicin (7 of 11 small tumors treated with the maximum tolerated dose of doxyrubicin showed a detectable decrease in growth, and two regressed completely) [16].

The tumor regression seen in these studies is particularly interesting because these compounds had been shown to be anti-proliferative, yet not overtly cytotoxic in cell culture. A strong cytostatic effect upon tumor cells could be enhanced by a depletion of angiogenic factors required to fully support tumor growth *in vivo*.

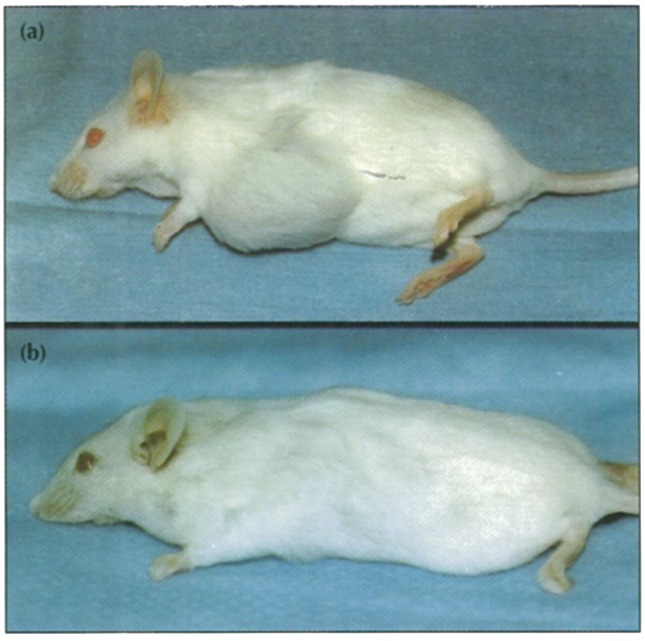


Fig. 3. Treatment of v-H-Ras-induced tumors with the peptidomimetic FTase inhibitor, L-744,832 (the propyl ester of L-739,750) leads to regression. **(a)** A mammary adenocarcinoma is clearly visible, despite 9 days of treatment with doxyrubicin. **(b)** After treatment of the same mouse with 40 mg kg^{-1} per day of L-744,832 for 27 days the tumor has regressed. Reprinted with permission from [16].

A recent report has shown that FTase inhibition causes a 50–90 % reduction in production of the angiogenic factor, vascular endothelial cell growth factor (VEGF) [17]. Despite the lack of visible remnants of the regressed tumors, if inhibitor treatment was stopped, tumors reappeared within two weeks. Although development of resistance is an important unresolved worry, it is promising that regrown tumors (two of three), and those which doxyrubicin had failed to control (three of five) remained sensitive to the inhibitor [16].

The mysteries of the mechanism

Although FTase inhibitors of very different types decrease growth of Ras-transformed cells, the specific mechanisms of growth inhibition remain unclear. FTase inhibitors would not be expected to restore normal function to an oncogenic Ras protein, yet tumor cell growth is inhibited with little effect on normal cells. It is possible that growth inhibition arises from the elimination of farnesylated oncogenic Ras, or that cytosolic accumulation of non-prenylated oncogenic Ras exerts a dominant negative effect [18]. Normal cells would remain unaffected as they lack oncogenic Ras. Recent results support the latter possibility; treatment of H-Ras-transformed cells with the prodrug of FTI-276 caused cytoplasmic accumulation of inactive H-Ras/Raf complexes, and blocked oncogenic activation of mitogen-activated (MAP) kinase [19], the eventual target of Raf. Successful disruption of oncogenic signaling by this mechanism would appear to depend upon a large amount and slow turnover of the cytosolic oncogenic Ras(GTP), which would complex significant amounts of Raf (Fig. 1). Conceivably, the rapid hydrolysis of GTP and the release of effectors by normal Ras proteins might protect normal cells from this effect. In fact, treatment of normal cells with BZA-5B caused no change in the EGF-induced activation of MEK and MAP kinases [20].

On the other hand, FTase inhibitors are not Ras-specific and also suppress modification of other farnesylated proteins [7, 11]. The nuclear lamin proteins are clearly farnesylated, and their farnesylation is blocked by inhibitors of FTase [21]. Inhibitors also cause dramatic effects on the morphology of transformed cells that do not correlate temporally with changes in Ras processing, suggesting that another farnesylated protein dominates the cytoskeletal responses to inhibitor [8]. The RhoB protein, which participates in control of the actin cytoskeleton, is one possibility, as it has been found to contain both C_{20} and C_{15} isoprenoids *in vivo* [22].

It is in the context of toxicity that the biggest surprises have emerged regarding FTase inhibitors. Despite evidence that inhibitors can enter normal cells and effectively prevent farnesylation of many proteins, normal cells show little change in growth rates. Remarkably, inhibitor-treated mice show no apparent ill effects on retinal, bone marrow, gastrointestinal tissues or even hair follicles [16], tissues that are known to contain farnesylated proteins or are dependent upon rapid growth and replacement of cells. Thus, in the systems tested so far, in which oncogenic Ras proteins have been overexpressed, it appears that growth of transformed cells or tumors can be inhibited at drug concentrations that leave the functions of normal cells intact.

The next step

These results point the way to the next important questions — what mechanisms are available to normal or transformed cells to compensate for the loss of farnesylation? Can GGTase I modify Ras proteins, either with its standard C_{20} or perhaps even unconventionally with a C_{15} isoprenoid? GGTase I, at least *in vitro*, can recognize proteins with a variety of residues in the X position and can transfer either C_{15} or C_{20} isoprenoid [2], with the choice depending on incompletely understood attributes of the protein substrate [22,23]. K-RasB may well be prenylated by GGTase I *in vivo*, as its carboxy-terminal polybasic domain and CVIM motif have been shown to permit such modification *in vitro*. A newly developed inhibitor (GGTI-287, the carboxy-terminal leucine derivative of FTI-276) shows five-fold specificity for GGTase I over FTase *in vitro*, and is more effective at blocking K-RasB prenylation and signalling in cells [24]. As most human tumors harbor a mutated form of the K-Ras gene [1], the participation of GGTase I in the prenylation of K-RasB [24], and the resistance of K-RasB to inhibition by BZA-2B [23] must be closely studied.

It is also possible that the loss of Ras farnesylation and function may not be as detrimental as we had previously

thought. It is already known that the loss of FTase activity by genetic knockout is not lethal to yeast [2]. Mammalian cells may possess proteins that act as surrogates for Ras when a normal cell's survival is at stake. The closest kin of Ras proteins, the R-Ras and R-Ras2/TC21 proteins, which are modified by C_{20} isoprenoids, could be such substitutes [25]. It is also possible that if inhibition of the FTase is incomplete, farnesylation and function of small amounts of Ras may be sufficient to allow survival of normal cells.

Finally, some intriguing results suggest that our ignorance of cellular isoprenoid metabolism may be greater than we had imagined. In recent studies in CHO-K1 cells [21], treatment with BZA-5B blocked incorporation of farnesyl moieties derived from [^{3}H]mevalonate into lamin B and prelamin A, and led to accumulation of nuclear prelamin A (as prenylation is required for the maturation of prelamin A). This treatment, however, had no effect upon assembly of the nuclear lamina, even when it was continued for 23 days. Why do these proteins continue to behave as if they were prenylated? It has been suggested [26] that the inhibitor-treated cells might be able to use a secondary pathway of isoprenoid metabolism or salvage in which isoprenoids not derived directly from [^{3}H]mevalonate are attached to proteins. Alternatively, these proteins might be modified through an unknown farnesylation-independent mechanism that allows maturation and function of proteins that would normally be farnesylated [21]. Confirmation of either possibility would have a profound impact on further development of FTase inhibitors, as both postulate the existence of as yet undiscovered enzymes that have been participating in protein prenylation. This raises the concern that Ras proteins may be able to regain tumorigenic activity if such alternate pathways become more active during treatment with FTase inhibitors.

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