Targeting LIM kinase in cancer and neurofibromatosis

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Neurofibromatosis type 1 is among the most common inherited diseases, affecting 1 in 3500 individuals. The phenotype of this autosomal-dominant disorder is highly variable and can be mild or severe. Symptoms might include café-au-lait spots, low intelligence, specific learning difficulties and behavioral problems, bone deformations, and various neurological syndromes. These patients are prone to a variety of benign and malignant tumors such as neurofibromas.

The tumor suppressor gene *Neurofibromin 1* encodes the 280-kDa Neurofibromin 1 protein (NF1). The latter's well-characterized Ras-GAP-related domain (GRD) facilitates GTP hydrolysis by Ras, thereby inactivating the Ras oncogene and its downstream pathways.[1] Loss of NF1 activity boosts Ras activity, encouraging cell proliferation. Our group reported that the high Ras.GTP phenotype of NF1-deficient cells could be partially reversed by the Ras inhibitor S-trans-farnesylthiosalicyclic acid (FTS, Salirasib), a Ras farnesylcysteine mimetic, with resulting inhibition of Ras downstream effectors including MAPK, PI3K-AKT, and Ral guanine nucleotide dissociation stimulator, leading, in turn, to reduced proliferation of $NF1^{-/-}$ cells and tumors.[2]

The link between neurofibromatosis and cancer is well established. While individuals bearing an NF1 mutation are at increased lifelong risk of developing cancer, mutations in NF1 were also recently found in many sporadic cancers. NF1 is now considered to be among the most frequently mutated tumor suppressors in colon, ovarian, and other cancers.

Does the tumor-suppressor activity of NF1 result only from inactivation of Ras? Recent findings suggest a new link between NF1 and cancer, via LIM kinases (LIMKs). The main substrate of these cytoskeleton regulators is the actin-depolymerizing factor protein cofilin, which is inactive when phosphorylated by LIMK. LIMK1 and 2 play important roles in pancreatic tumor progression, cancer cell-induced angiogenesis, and metastasis formation.[3]

LIMK2 apparently promotes cancer cell survival and contributes to chemotherapy resistance in neuroblastoma cell lines and p53-mediated survival of DNA-damaged cancer cells.[4] Recent findings that LIMK2 is directly activated by Aurora A and promotes its regulation suggest that LIMK2 inhibition might inhibit Aurora A-mediated oncogenic pathways[5] (**Fig. 1**).

NF1, as an upstream regulator of LIMK1/2, can inactivate these proteins in several ways: its GRD enhances cell motility by regulating actin-filament dynamics via the Rho-ROCK-LIMK2-cofilin pathway; its pre-GRD region negatively regulates the Rac1-Pak1-LIMK1-cofilin pathway (reviewed in ref. 3); its SecPH domain directly binds and inhibits activation of ROCK and specifically Rock activation of LIMK2, providing novel crosstalk between NF1 and small-GTPase regulation[6] (**Fig. 1**).

We recently designed a novel LIMK inhibitor by combining different computational methods and used classical biochemistry techniques to identify, characterize, and validate our compound as a novel anti-cancer drug.[7] The MODLER algorithm predicted structural similarity between the LIMK2 kinase domain and the substrate-binding site of EphA3. Based on this similarity, we predicted that EphA3 inhibitor would fit into LIMK2 substrate binding pocket and ATP-binding site and will inhibit LIMK phosphorylation of cofilin phosphorylation of cofilin by LIMK. Our novel compound, T56-LIMKi (3-methyl-*N*-[3-{(3-[trifluoromethyl] phenyl) carbamoyl} phenyl] isoxazole-5-carboxamide), like the EphA3 inhibitor it resembles, inhibited LIMK2 with high specificity but hardly crossreacted with LIMK1. In several cancer cell lines, including those of pancreatic cancer, glioma and shwannoma,[7,8] T56-LIMKi blocked phosphorylation of cofilin, leading to actin severance, inhibition of tumor cell growth and migration, and anchorage-independent colony formation in soft agar.

The most promising in vitro effect of T56-LIMKi was observed in the pancreatic cancer cell line Panc-1. T56-LIMKi also reduced tumor size and p-cofilin levels in Panc-1 tumors in a nude mouse Panc-1 xenograft model, leading us to propose this inhibitor as a candidate drug for cancer therapy.[8]

Since LIMK2 is known to be hyperactive in neurofibromatosis type 1, we examined the effect of T56-LIMKi on NF1-depleted mouse embryonic fibroblasts (NF-1$^{-/-}$ MEFs). T56-LIMKi indeed inhibited cell growth, actin stress-fiber formation, cell migration, and colony formation in soft agar of NF-knockout cells.[7]
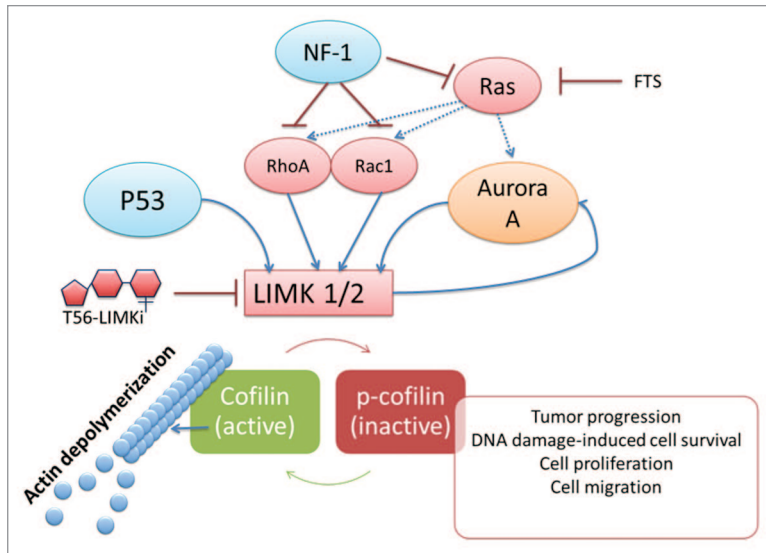
Since LIMK modulation by NF1 is not affected by the Ras inhibitor FTS, we examined the combined effect of FTS and T56-LIMKi, which affect cell motility through distinct pathways. Their

Figure 1. LIM kinases tumor progression function is tightly regulated by major oncogene and tumor supressors. The actin-depolymerizing factor (ADF)/cofilin family of proteins plays a prominent role in promoting actin depolymerization. Cofilin is phosphorylated mainly by LIMK1 and LIMK2. In its phosphorylated state, cofilin is inactive. The unphosphorylated active cofilin induces severing of actin filaments and participates in many cellular functions critical for normal and cancer cells, such as cell migration, cell cycle, and neuronal differentiation. LIMK activation is regulated by Rho family GTPases, which are themselves targets for Ras activity. In general, LIMK2 is activated by Rho, whereas LIMK1 is activated by Rac1. Neurofibromin 1 (NF1) regulates LIMK activity through several pathways: its GRD domain inhibits the Ras-dependent pathway and independently also inhibits a Rho-dependent pathway, which activates LIMK2; the pre-GRD domain inhibits the Ras-independent Rac1-activation pathway, which regulates LIMK1; and NF1 also directly inhibits ROCK-induced phosphorylation of LIMK2. LIMK2 is a cancer cell-survival factor that mediates p53-mediated survival of cancer cells following DNA damage. In addition, LIMK2 is directly activated by Aurora A and is a key oncogenic effector of this kinase. We suggest that inhibition of LIMK2 has promising therapeutic potential for cancer cells characterized by LIMK2 overactivation or by overactivation of LIMK2 upstream signaling pathways such as those of RhoA, p53, and Aurora A kinase.

or overactivation of LIMK2 upstream signaling pathways such as RhoA, and in cells with low NF1 expression, we think that appropriate selection of cancer properties for targeting is crucial to the success of this proposed treatment. As cancer treatment becomes increasingly personalized, with molecular assays capable of quantitating genes, proteins, or specific mutations in tumors, the demand for specifically targeted biological drugs will increase. As a selective drug for LIMK2 inhibition, T56-LIMKi will be helpful for cancer treatment with and without classical radio- and chemotherapy (especially cell cytoskeleton inhibitors such as Vincristine and Taxol), or in combination with other biological cancer drugs (e.g., the Ras inhibitor FTS or Aurora A inhibitors such as alisertib).

References

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combined action on cell proliferation and stress-fiber formation in NF1^{-/-} MEFs was synergistic.[7] We suggest that this drug combination may be developed for treatment of neurofibromatosis type 1 and cancer.

Since the inhibitor was more effective in cells displaying LIMK2 overactivation[8]