

The Importance of Isoprenylation and *Nf1* Deficiency in K-RAS–induced Cancer

Anna-Karin Sjögren

*The Wallenberg Laboratory, Department of Molecular and Clinical Medicine,
Institute of Medicine at Sahlgrenska Academy, University of Gothenburg, Sweden*

The RAS and RHO family proteins contribute to tumorigenesis and metastasis and belong to a family of so called CAAX proteins. The membrane targeting and proper function of CAAX proteins are dependent on posttranslational isoprenylation by farnesyltransferase (FTase) or geranylgeranyl-transferase type I (GGTase-I). Inhibitors of FTase and GGTase-I have been developed to block RAS-induced cancer, but their utility has been difficult to evaluate because of off-target effects, drug resistance, and toxicity. One aim of this thesis was to use genetic strategies in mice to define the physiologic importance of CAAX protein isoprenylation and to evaluate FTase and GGTase-I as potential anti-cancer drug targets.

Oncogenic mutations in RAS are common in cancer and result in hyperactive RAS signaling. However, a RAS mutation alone is not sufficient for cancer development in humans. Rather, cancer arises as a consequence of cooperation between several mutational events. The tumor suppressor gene neurofibromatosis type I (*NF1*) is a RAS-inactivating protein. Thus, loss of *NF1* also results in hyperactive RAS signaling and this occurs in some types of cancer. It has been proposed that *NF1* deficiency is functionally equivalent to an oncogenic RAS; but *NF1* may operate in other pathways. It is not clear if *NF1* deficiency would be redundant in RAS-induced cancer development or if the two mutations would cooperate. A second aim of this thesis was to define the impact of *Nf1* deficiency on the development of K-RAS–induced cancer in mice.

To approach these aims, Cre/*loxP* gene targeting techniques in mice were used, to simultaneously activate an oncogenic K-RAS allele, to induce lung cancer or myeloid leukemia, and inactivate the genes encoding FTase and GGTase-I, or *Nf1*.

Inactivating the gene encoding the β -subunit of GGTase-I eliminated enzyme activity, blocked proliferation and reduced motility of fibroblasts. Moreover, inactivation of GGTase-I reduced tumor formation and increased survival of mice with K-RAS–induced lung cancer. Finally, several cell types, including lung tumor cells and macrophages remained viable in the absence of GGTase-I.

Inactivating the gene encoding the β -subunit of FTase eliminated farnesylation of HDJ2 and H-RAS, prevented H-RAS targeting to the plasma membrane, and blocked proliferation of fibroblasts. FTase inactivation reduced tumor formation and increased survival of mice with K-RAS–induced cancer to a similar extent as the inactivation of GGTase-I. The simultaneous inactivation of FTase and GGTase-I markedly reduced lung tumors and improved survival.

These data suggest that inhibition of FTase and/or GGTase-I could be useful in the treatment of K-RAS–induced cancer.

In mice, expression of oncogenic K-RAS or inactivation of *Nf1* in hematopoietic cells results in myeloproliferative disorders (MPDs) that do not progress to acute myeloid leukemia (AML). However, the simultaneous inactivation of *Nf1* and activation of oncogenic K-RAS in hematopoietic cells induced AML in mice. The levels of active RAS were not increased in mice with AML, raising the possibility that *Nf1* deficiency may contribute to AML by non-RAS pathways.

This result points to a strong cooperation between *Nf1* deficiency and oncogenic K-RAS and sheds new light on mechanisms of RAS-induced leukemia development.

Keywords: FTase, GGTase-I, isoprenylation, K-RAS, NF1, CAAX proteins, lung cancer, AML

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Anna-Karin Sjögren

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I. GGTase-I deficiency reduces tumor formation and improves survival in mice with K-RAS–induced lung cancer

Sjögren AK, Andersson KM, Liu M, Cutts BA, Karlsson C, Wahlstrom AM, Dalin M, Weinbaum C, Casey PJ, Tarkowski A, Swolin B, Young SG, Bergo MO.

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II. Targeting the protein prenyltransferases efficiently reduces tumor development in mice with K-RAS–induced lung cancer

Liu M*, Sjögren AK*, Karlsson C, Ibrahim M, Andersson KM, Olofsson FJ, Wahlstrom AM, Dalin M, Yu H, Yang S, Young SG, Bergo MO.

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*These authors contributed equally.

III. *Nf1* deficiency cooperates with oncogenic K-RAS to induce acute myeloid leukemia in mice

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*These authors contributed equally.



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