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1994 - 1997	PostDoc - Institute for Biochemistry I, University of Heidelberg, Germany (Prof. Felix Wieland)
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Unconventional Protein Secretion

Goal

To reveal the molecular components and mechanisms involved in unconventional secretion of fibroblast growth factor 2 from tumor cells as well as to identify small molecule inhibitors of this process to develop a novel class of anti-angiogenic drugs.

Background

The vast majority of extracellular proteins is secreted through the classical ER/Golgi-dependent secretory pathway, however, numerous exceptions have been identified. As opposed to proteins that are transported along the classical route, unconventional secretory proteins lack a signal peptide and their export from cells is not affected by brefeldin A, an inhibitor of ER-to-Golgi trafficking. Several kinds of unconventional secretory pathways have been described some of which involve intracellular vesicles such as secretory lysosomes or multi-vesicular bodies. By contrast, unconventional secretion of fibroblast growth factor 2 (FGF2) has been shown to occur by direct translocation across plasma membranes resulting in its association with heparan sulfate proteoglycans on cell surfaces. Using biochemical reconstitution experiments and genome-wide RNAi screening approaches, our laboratory aims at a functional dissection of the unconventional secretory pathway of FGF2 at the molecular level. We further make use of these insights to develop small molecule inhibitors as lead compounds for the development of novel drugs that inhibit tumor-induced angiogenesis as well as prevent anti-apoptotic effects of FGF2 that cause resistance of tumor cells against chemotherapy.

Research Highlights

In recent years, we have revealed a number of key steps of the molecular mechanism by which FGF2 translocates across the plasma membrane to reach the extracellular space (Fig. 1). Following the initial observation that FGF2 can physically traverse the plasma membrane, we identified a binding pocket for the phosphoinositide $PI(4,5)P_2$ in the crystal structure of FGF2. Further studies revealed that FGF2 secretion is initiated by $PI(4,5)P_2$-dependent recruitment of FGF2 at the inner leaflet of the plasma membrane. This process causes FGF2 to homo-multimerize driving membrane insertion of a FGF2 oligomer that has been hypothesized to have a ring-like, membrane-pore-forming structure. This view is supported by reconstitution experiments with model membranes where $PI(4,5)P_2$-dependent oligomerization and membrane insertion of FGF2 causes both membrane passage of small fluorescent tracers and transbilayer diffusion of

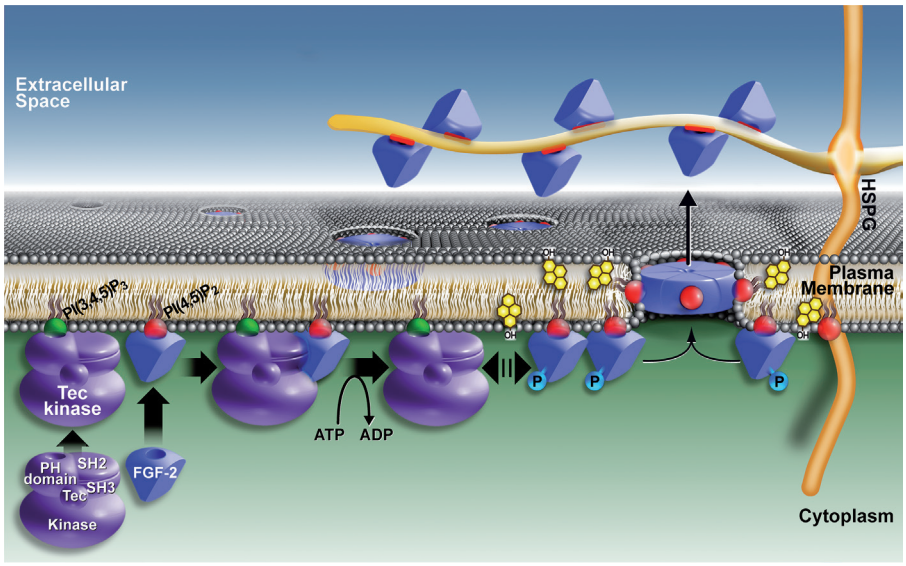

Fig. 1: *Molecular components and mechanisms involved in unconventional secretion of FGF2 from tumor cells.*

membrane lipids. Based on these observations, a toroidal architecture of lipidic membrane pores formed by FGF2 oligomers was proposed.

Membrane insertion of FGF2 oligomers is strongly enhanced by Tec-kinase-mediated tyrosine phosphorylation of FGF2, a potential regulatory mechanism that controls FGF2 secretion. This view is further supported by cell-based assays with Tec kinase being required for efficient FGF2 secretion from cells. Intriguingly, Tec kinase contains a PH domain mediating its recruitment to the inner leaflet of the plasma membrane in a $PI(3,4,5)P_3$ dependent manner. This may in part explain why PI3 kinases, the enzymes that synthesize $PI(3,4,5)P_3$, are up-regulated in many cancers.

Our findings point to a role of membrane-inserted FGF2 oligomers as translocation intermediates in unconventional secretion of FGF2. To translocate FGF2 to the cell surface, membrane-inserted oligomers of FGF2 need to be removed from the membrane, a process that was shown to be mediated by cell surface heparan sulfate proteoglycans that compete against $PI(4,5)P_2$ for binding to FGF2. These findings have been interpreted as an extracellular trapping mechanism resulting in cell surface exposure of FGF2. The overall process of FGF2 secretion therefore relies on sequential interactions of FGF2 with $PI(4,5)P_2$ and Tec kinase at the inner leaflet, membrane pore formation by FGF2 oligomerization and extracellular trapping mediated by cell surface heparan sulfate proteoglycans.

In addition to the molecular characterization of further components of the secretory machinery of FGF2, a major focus of our laboratory is now to identify small molecule inhibitors of FGF2 secretion. In a first attempt, we have identified inhibitors of the interaction of FGF2 and Tec kinase. Intriguingly, such compounds inhibit both phosphorylation of FGF2 and secretion of FGF2 from cells. It is a key aim to expand these efforts to all known components of FGF2 secretion to achieve our long-term goal of the development of anti-cancer drugs that are based on the inhibition of FGF2 release from tumor cells.

Selected Publications 2011 - 2013

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