# Nikolaus-Fiebiger-Center of Molecular Medicine

## Chair of Experimental Medicine II (Molecular Oncology)

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**Research focus**
- Molecular oncology of Wnt signaling
- Amer proteins
- Role of Axin/Conductin as negative Wnt regulators

## Structure of the Chair

Professorship: 1
Personnel: 9
- Scientists: 3 (thereof funded externally: 0)
- Graduate students: 2

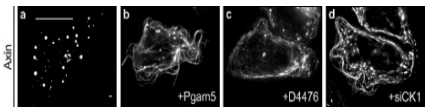
**Special structural feature**
Managing Director of the Nikolaus-Fiebiger-Center (NFZ) alternating biannually with Chair of Experimental Medicine I

## Research

The focus of research is on the molecular analysis of signal transduction pathways causally involved in tumor diseases. Over the last years, central components of the oncogenic Wnt signaling pathway were identified through special screening approaches and analyzed in molecular detail. These efforts have contributed to the identification of novel targets for therapy aimed at inhibition of the pathway, which are currently intensively investigated worldwide.

**Molecular oncology of Wnt signaling**
The Wnt signaling pathway controls the stability of β-catenin and thereby regulates various processes during embryonic development and can lead to cancer. Wnt are secreted glycoproteins, which induce the accumulation of β-catenin in cytoplasm and nucleus by binding to frizzled and LRP receptors. β-Catenin interacts with TCF transcription factors and activates target genes. The destruction of β-catenin is induced by phosphorylation in a multi-protein complex consisting of the scaffold components axin or conductin, the serine/threonine kinase GSK3β, and the tumor suppressor APC (Adenomatous Polyposis Coli). The Wnt signal inhibits phosphorylation of β-catenin and thereby leads to its stabilization. In colorectal tumors, mutations of APC or of the serine/threonine phosphorylation sites of β-catenin lead to stabilization of β-catenin and trigger constitutive signaling to the nucleus. Such β-catenin mutations are also found in a multitude of other tumor types suggesting that aberrant activation of Wnt signaling is a key mechanism of oncogenic transformation. We are analyzing the molecular roles of central components of the pathway, which are mostly involved in β-catenin degradation. Among these are Amer1, Axin, Conductin as well as the phosphatase PGAM5 that all modulate β-catenin phosphorylation.

**Role of Axin/Conductin as negative Wnt regulators**
PI: Dr. D. Bernkopf
Axin and conductin (also known as axin2) are structurally related inhibitors of Wnt/β-catenin signaling that promote degradation of β-catenin. Whereas axin is constitutively expressed, conductin is a Wnt target gene implicated in Wnt negative-feedback regulation. By proteome analysis we could demonstrate an interaction of axin with the mitochondrial phosphatase PGAM5. PGAM5 gets cleaved and released to the cytoplasm after damage of the mitochondrial membrane potential. Cytoplasmic PGAM5 interacts with axin, and the resulting complex induces dephosphorylation of β-catenin leading to its stabilization and activation of β-catenin-dependant transcription. Since Wnt signaling is known to increase mitochondrial numbers, we proposed that the release of cytoplasmic PGAM5 from damaged mitochondria induces formation of new mitochondria by activating the Wnt pathway (Bernkopf et al., 2018). In the course of these studies we noticed that Pgam5 alters the localization pattern of axin. Whereas transiently expressed axin formed the typical axin puncta, co-expression of PGAM5 frequently induced the formation of long elongated axin polymers resembling fibrils (Fig. 1, a,b). Importantly, inhibition or knockdown of a central kinase in the Wnt/β-catenin signaling pathway, casein kinase 1 (CK1) also induced the formation of axin fibrils (Fig. 1c, d), suggesting that endogenous CK1 controls the axin polymerization mode. These data revealed the existence of a yet uncharacterized fibrillary form of axin polymers besides the well-studied punctate form, and show that the transition between both is a regulated process involving phosphorylation. Of note, Pgam5-induced axin fibrils co-localized with microtubules (MTs) and were destroyed by depolymerization of MTs with nocodazole (not shown).
We also noticed that puncta of transiently expressed axin as well as of endogenous axin were in close proximity and appeared associated to MTs These data suggest that MTs provide a platform for fibril formation by axin, and might be involved in degrading β-catenin, which is suggested by preliminary data of our group. Although conductin is strongly upregulated in colorectal cancer as a target of Wnt signaling, its activity apparently does not suffice for blocking β-catenin. We noticed that conductin per se is less active that axin in degrading β-catenin. We found that axin and conductin differ in their intracellular distribution with axin polymerizing into microscopically-visible puncta while conductin was distributed diffusely all over the cytoplasm. This became of functional interest because axin-mediated β-catenin degradation depends on polymerization. By exchanging domains between both homologs, we could map the differential distribution to the regulator of G-protein signaling (RGS) domain (Bernkopf et al., 2019).
We discovered a predicted aggregation site in the RGS domain of conductin that is lacking in the RGS domain of axin and showed that RGS mediated aggregation blocks conductin polymerization. Importantly, inactivation of this aggregon by specific amino acid mutation allowed polymerization of conductin and led to increased activity of conductin in degrading β-catenin. Together, these data suggest that interfering with RGS-mediated aggregation promotes DIX-mediated polymerization of conductin and inhibits Wnt signaling. In order to develop a strategy to promote conductin polymerization, we designed short peptides containing the aggregation site, which would compete with RGS-RGS aggregation of conductin. Indeed, co-expression of such a peptide induced polymerization of conductin, reduced Wnt signaling and suppressed growth of colorectal cancer cells in vitro (Bernkopf et al., 2019).
We are currently optimizing the peptide for in vivo use and plan to screen chemical libraries of FDA-approved small-molecules for compounds inducing conductin polymerization. These could serve as peptide alternatives and potential chemotherapeutic drugs in the future.




*Puncta of axin (a), and fibril formation after Pgam5 expression (b), CK1 inhibition (c), or CK1 knockdown (d).*

## Teaching

The Chairs of Experimental Medicine I and II are primarily responsible for the training of bachelor and master students of Molecular Medicine in cell biology and molecular oncology.
Bachelor's and Master's theses are supervised.

**Selected publications**
Bernkopf DB, Jalal K, Bruckner M, Knaup KX, Gentzel M, Schambony A, Behrens J. Pgam5 released from damaged mitochondria induces mitochondrial biogenesis via Wnt signaling. J Cell Biol 2018, 217, 1383-1394

Bernkopf DB, Brückner M, Hadjihannas MV, Behrens J. An aggregon in conductin/axin2 regulates Wnt/β-catenin signaling and holds potential for cancer therapy. Nat Commun. 2019, 18; 4251.

**International cooperation**
Prof. V. Katanaev, University Geneva, Geneva: Switzerland