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Thesis Abstract

"A good means to discovery is to take away certain parts of a system and to see how the rest behaves." – Georg Christoph Lichtenberg (1742-1799)

Extensive studies using kinase inhibitors or overexpression of dominant negative mutants have demonstrated a pivotal role for class Ia phosphoinositide 3-kinase (PI3K) in carcinogenesis and glucose homeostasis as it regulates key cellular processes such as proliferation, survival, motility and glucose uptake. Class Ia PI3K is composed of a regulatory subunit, p85, and a catalytic subunit, p110. Various isoforms have been described for both subunits. The early embryonic death of mice deleted for PI3K catalytic isoforms (p110α-/- mice and p110β-/- mice) points to isoform specific functions since the remaining isoform cannot compensate for the deleted one. Furthermore, microinjection of inhibitory antibodies to the catalytic activity of PI3K has implied that mainly p110α mediates PDGF signals *in vitro*, while specifically the p110β isoform might transduce insulin signals. It is not clear how this specificity might be achieved. One possibility might be that specificity is derived from the PI3K regulatory isoforms.

Here, I took a genetic approach to dissect specific functions of both the regulatory and catalytic isoforms of class Ia PI3K *in vivo*. To do so, I analyzed mice lacking PI3K regulatory or catalytic isoforms. We determined that only combined loss of p85α and p85beta gene products (with or without intact expression of the smaller isoforms p55α and p50α) results in early embryonic lethality. We could see that in contrast to p110α and p110β which fulfill isoform specific functions during embryonic development, the PI3K regulatory isoforms p85α and p85β act in a redundant manner. The smaller isoforms p55α and p50α can partially compensate for the loss of 85kD isoforms. Interestingly, combined loss of p85α and p85β as well as p110α targeted disruption results in defects similar to those seen in PDGF receptor α -/- mice. Intact expression of p55α and p50α does not rescue the mice. We therefore conclude that during development PDGF receptor alpha transmits signals specifically through p110α/p85α or p110α/p85β complexes.

Since mice deleted for all p85α and p85β gene products (p85α-/-p55γ-/-p50α-/-p85β-/- mice) show defects similar to mice lacking PDGF receptor β, I established cell lines to further examine PI3K isoform specific functions in PDGF signaling *in vitro*. Results presented in chapter 3 show defects in PDGF induced lamellipodia formation and cell migration upon loss of the p85 subunit. Retroviral reintroduction of p85α, p85β or p50α restores the ruffling defect showing that the Rho-GAP domain, which is lacking in the small PI3K regulatory isoform p50α, is not necessary for PDGF induced lamellipodia formation.

In addition, in chapter 4, I investigate whether loss of p110 catalytic subunits of PI3K causes a similar increase in insulin sensitivity to that seen in mice lacking p85 subunits. Since p110α- or p110β-null mice are embryonic lethal, I analyzed mice with heterozygous loss of p110α and/or p110β. Interestingly, p110α+/-p110β+/- mice exhibit decreased p110 as well as p85 protein levels and a tendency to insulin resistance. Given that loss of any PI3K regulatory isoform results in hypersensitivity to insulin, my findings show that insulin signaling is controlled by a tight balance between the negative regulator, p85, and a positive regulator, p110.

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