Receptor tyrosine kinase signalling in the absence of growth factor stimulation

Chi-Chuan Lin, Jonathan Berrout, Arndt Rohwedder, Janne Darell and John Ladbury

Overview of research

In the absence of extracellular stimulation or genetic mutation, an oncogenic response can be driven by the competitive binding of SH3 domain-containing downstream effector proteins to proline-rich sequences on growth factor receptors. Of the approximately 50 plasma membrane receptor tyrosine kinases (RTKs) the majority have proline-rich sequences in their C-termini. These have a propensity to bind to the >300 proteins expressed in human cells which contain SH3 domains. These interactions occur in the absence of any extracellular stimulation (e.g. growth factors, cytokines). Proline-rich sequence binding to SH3 domains are promiscuous and the observed interactions with RTKs are dependent on the relative concentrations of the proteins involved.

We previously established that under non-stimulatory conditions the fibroblast growth factor receptor 2 (FGFR2) recruits the adaptor protein, growth factor receptor binding protein 2 (Grb2) through its C-terminal SH3 domain. In cells depleted of Grb2 other proteins can access the proline-rich motif on FGFR2. One of these proteins, phospholipase C(gamma)1 (Plcγ1) is activated on binding and through turnover of plasma membrane phospholipids to produce second messengers, raises cellular calcium levels which are responsible for increased cell motility and invasive behaviour. In ovarian and lung adenocarcinoma patients with low levels of Grb2 and increased expression of Plcγ1higher incidence of metastasis leads to greatly reduced survival outcomes.

We have extended our studies in this area to explore other RTK-SH3 domain-containing protein interactions to establish whether the up-regulation of signal transduction through these interactions is a general phenomenon. This leads to the hypothesis that two tiers of intracellular signalling can be derived from receptors with intrinsic protein kinase activity:
1) Ligand-induced elevation in kinase activity resulting in tyrosylphosphate-mediated effector protein recruitment and committal to a defined cellular outcome (e.g. proliferation).
2) Receptor phosphorylation-independent activation of downstream effectors through SH3 domain/proline-rich sequence interactions, which appear to be required for cell homeostasis/metabolic control.

Hyperactivity of the tier 1 signalling is a feature of receptor tyrosine kinase-related cancers arising from genetic mutation. Although the tier 2 signalling mechanism occurs under basal conditions, and is thus likely to be associated with cellular maintenance, we have shown that fluctuations in expression levels of SH3-containing proteins can drive cells into pathological phenotypes including proliferation and metastasis.

We are testing this hypothesis with a range of methods extending from cell-based assays (including fluorescence lifetime imaging microscopy) through to structural and in vitro biophysical analysis.

Publications

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