

Protein Kinases and Disease in the Postgenomic Era

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Protein kinases and phosphatases are key components of protein phosphorylation based signaling networks in eukaryotic cells. In consequence, there has been long-standing interest in the number of protein kinases (PKs) and phosphatases (PPs) needed to constitute intracellular signaling networks. We have used complete genome sequences combined with cDNA and EST sequence information to determine the total number of PK genes (the kinome) in budding yeast, *C. elegans*, *Drosophila*, mouse and *Homo sapiens* (1, 2). The human kinome has 518 PK genes; 478 of these PKs belong to the eukaryotic PK (ePK) superfamily, and the remainder are atypical PKs (aPK), divided among a few small families. Over 50 of the ePK catalytic domains in the human kinome are missing one or more of the key ePK catalytic residues, and are therefore predicted to lack catalytic activity; these domains presumably have functions other than phosphate transfer. Comparison of the kinomes from different eukaryotic species provides insights into the evolutionary history of the PKs. PKs constitute ~2% of all genes in budding and fission yeast, worms, flies and humans, and ~4% in plants. True tyrosine kinases (TKs) are lacking in single cell yeasts, but are present in simple metazoans, suggesting that tyrosine phosphorylation evolved as a mechanism for intercellular communication. In metazoans 15-20% of all PKs are TKs. Mutations in PK and PP genes are increasingly found to be causal in human diseases; out of 518 PK genes ~150 have been implicated in disease (3, 4). Activating (or more rarely inactivating) mutations, or overexpression of 116 tyrosine and serine kinases has been associated with human cancer. The prevalence of PKs involved in disease has led to intensive efforts to develop specific PK inhibitors and major efforts are underway in the pharmaceutical industry and academia to develop PK inhibitors as cancer therapeutics and treatments for other diseases. Five tyrosine kinase inhibitors (TKIs) (Gleevec, Iressa, Tarceva, Sutent, Sprycel) and one serine/threonine/tyrosine kinase inhibitors (Nexavar) are approved for clinical use in cancer therapy in the US; >70 other PK inhibitors are in cancer clinical trials, and >25 PK inhibitors are in trials for diseases other than cancer. In addition, rapamycin, an mTOR inhibitor, and analogues are also in clinical trials for several cancers.

Tuberous sclerosis is a largely benign tumor syndrome resulting from the acquisition of somatic lesions in genes encoding the tumor suppressor proteins, TSC1 or TSC2. Loss of function of the TSC1-TSC2 complex, which acts as a Rheb GAP, yields constitutive, unrestrained signaling from the cell growth machinery comprised of the small G protein Rheb, and the mTOR aPK and S6K ePK. We have found that constitutive activation of the Rheb/mTOR/S6K cassette, either through genetic deletion of TSC1 or TSC2 or by ectopic expression of Rheb, is sufficient to induce insulin resistance (5). This is due to a ~90% decrease in the levels of the insulin receptor substrates, IRS1 and IRS2, which are critical for signaling downstream of the insulin receptor and which become limiting for activation of PI-3 kinase in response to insulin in TSC-deficient cells. Downstream of PI-3K, the survival kinase, Akt, is completely refractory to activation by insulin and IGF-I IRS-dependent growth factor pathways in TSC1/2-deficient fibroblasts. In consequence, the anti-apoptotic program induced by IGF-I is severely compromised in TSC2-null cells. These effects are reversed by treatment with the mTOR inhibitor rapamycin, which increases IRS1/2 protein levels over several hours. The feedback loop that attenuates insulin receptor signaling requires S6K activity, and this involves direct phosphorylation of IRS1 by S6K at multiple RXXRXXS sites, which prevents IRS1 association with the activated insulin receptor, results in dissociation of IRS1 from an active signaling compartment, and also in an increased rate of IRS1 protein degradation. In addition, there is decreased transcription of the *IRS1* and *IRS2* genes in TSC-deficient cells. Our results suggest that inappropriate activation of the Rheb/mTOR/S6K pathway through prolonged insulin stimulation or genetic lesions imposes a negative feedback program to attenuate IRS-dependent processes, such as cell survival. In addition, human cancers commonly exhibit upregulated mTOR signaling and this leads to

activation of the S6K feedback loop and downregulation of Akt activity. In consequence, the use of rapamycin for cancer therapy, which is now being tested in the clinic, may be less effective than anticipated because it will reactivate PI3K/Akt survival signaling in these cells, and rapamycin might be a more effective as a cancer therapy if used in combination with inhibitors of upstream regulators of Akt (e.g. the IGF-1 receptor or PI-3K, or Akt itself).

We have also found that TSC1 and TSC2-null mouse embryo fibroblasts (MEFs) are hypersensitive to treatment with DNA damaging agents, including chemotherapeutic drugs like doxorubicin, with the TSC-null cells undergoing rapid apoptosis, thus implicating TSC1/TSC2 in cell survival responses. From our earlier work, we knew that Akt survival signaling was deficient in TSC-null cells, but Akt is not activated by DNA damage in MEFs. However, we found that DNA damage-mediated activation of another survival pathway, namely NF- κ B, which induces the expression of many genes including a program of cell survival genes, is severely depressed in TSC-null cells (6). Reconstitution of TSC2 expression in TSC2-null MEFs restores cell survival in response to DNA damage, in a manner that requires NF- κ B activity, indicating that NF- κ B functions as an important survival pathway in TSC2-dependent cell survival. Furthermore, treatment of TSC2-null MEFs with rapamycin, an mTOR inhibitor, restores NF- κ B activation and promotes survival. This rapamycin-mediated effect is reversed by inhibition of NF- κ B transcriptional activation or by inhibition of ERK1/2 MAP kinase or PI-3K pathways, which lie on signaling cascades leading to NF- κ B activation. Our results suggest that there is unexpected crosstalk between the TSC/Rheb/mTOR pathway and NF- κ B induction pathways that modulates NF- κ B activation and cell survival. Rapamycin has been suggested as a potential therapeutic reagent for a wide variety of cancers with mutations that activate the PI-3 kinase-TSC1/2-mTOR signaling pathway, possibly being used in combination with chemotherapeutic drugs. However, since rapamycin treatment increases resistance of TSC-deficient cells to chemotherapeutic drugs and since this can be reversed by inhibition of NF- κ B activation or its upstream signaling pathways, our studies suggest that NF- κ B signaling inhibitors (e.g. an IKK inhibitor) could be used as adjuvants to maximize the efficacy of rapamycin-based therapeutics for TSC-derived tumors or for other types of cancer with hyperactive mTOR signaling.

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