ONCOGENE

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Guest Editor **S. Sebti**

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The rapamycin-sensitive signal transduction pathway as a target for cancer therapy

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The high frequency of mutations in cancer cells which result in altered cell cycle regulation and growth signal transduction, conferring a proliferative advantage, indicates that many of these aberrant mechanisms may be strategic targets for cancer therapy. The macrolide fungicide rapamycin, a natural product with potent antimicrobial, immunosuppressant, and anti-tumor properties, inhibits the translation of key mRNAs of proteins required for cell cycle progression from G_1 to S phase. Rapamycin binds intracellularly to the immunophilin FK506 binding protein 12 (FKBP12), and the resultant complex inhibits the protein kinase activity of a protein kinase termed mammalian target of rapamycin (mTOR). The inhibition of mTOR, in turn, blocks signals to two separate downstream pathways which control the translation of specific mRNAs required for cell cycle traverse from G_1 to S phase. Blocking mTOR affects the activity of the 40S ribosomal protein S6 kinase ($p70^{s6k}$) and the function of the eukaryotic initiation factor 4E-binding protein-1 (4E-BP1), leading to growth arrest in the the G_1 phase of the cell cycle. In addition to its actions on $p70^{s6k}$ and 4E-BP1, rapamycin prevents cyclin-dependent kinase activation, inhibits retinoblastoma protein (pRb) phosphorylation, and accelerates the turnover of cyclin D1 that leads to a deficiency of active cdk4/cyclin D1 complexes, all of which can inhibit cell cycle traverse at the G_1/S phase transition. Both rapamycin and CCI-779, an ester analog of rapamycin with improved pharmaceutical properties and aqueous solubility, have demonstrated impressive activity against a broad range of human cancers growing in tissue culture and in human tumor xenograft models, which has supported the development of compounds targeting rapamycin-sensitive signal-transduction pathways. CCI-779 has completed several phase I clinical evaluations and is currently undergoing broad disease-directed efficacy studies. The agent appears to be well tolerated at doses that have resulted in impressive anti-tumor activity in several types of refractory neoplasms. Important challenges during clinical development include the definition of a recommended dose range associated with optimal biological activity and maximal therapeutic indices, as well as the ability to predict which tumors will be sensitive or resistant to CCI-779. *Oncogene* (2000) **19,** 6680–6686.

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Cell proliferation is a complex multifaceted process that requires the synthesis of essential regulatory proteins involved in the transduction of extracellular and autocrine proliferative stimuli. Since several of these highly regulated processes are aberrant in many types of cancers, conferring a proliferative advantage, they are potential strategic targets for therapeutic development against cancer (Sherr, 2000). Indeed, several novel classes of therapeutics that interfere with discrete essential elements of aberrant signal transduction and cell cycle regulation, such as inhibitors of various receptor tyrosine kinases, oncogenes, critical proteins involved in signal transduction (e.g. Ras, Raf), and cyclin-dependent kinases, are being developed as anti-cancer agents (Rowinsky *et al*., 1999, Senderowicz and Sausville 2000). One such agent, rapamycin (sirolimus; Rapamune®; Wyeth-Ayerst, PA, USA), a macrolide fungicide isolated from the bacteria *Streptomyces hygroscopicus*, possesses potent antimicrobial, immunosuppressant, and antitumor properties (Baker *et al*., 1978; Sehgal *et al*., 1975; Vezina *et al*., 1975). Because of its profound immunosuppressive actions, rapamycin was initially developed and received regulatory approval for the indication of prevention of allograft rejection following organ transplantation (Sehgal, 1995). The antiproliferative actions of rapamycin have been demonstrated to be due to its ability to modulate critical signal transduction pathways that link mitogenic stimuli to the synthesis of proteins required for cell cycle traverse from G_1 to S (Wiederrecht *et al*., 1995). Impressive antiproliferative activity has been demonstrated following treatment of a diverse types of experimental tumors with rapamycin (Eng *et al*., 1984, Muthukkumar *et al*., 1995; Seufferlein and Rozengurt, 1996). However, the poor aqueous solubility and chemical stability of rapamycin precluded its clinical development as an anti-cancer agent. Recently, a series of rapamycin analogs with improved aqueous solubility and stability have been synthesized and evaluated. CCI-779 (Wyeth Ayerst, PA, USA), a soluble ester analog of rapamycin, was selected for development as an anti-cancer agent based on its prominent anti-tumor profile and favorable pharmaceutical and toxicological characteristics in preclinical studies (Gibbons *et al*., 2000). Several phase I studies of CCI-779 have been completed and disease-directed efficacy evaluations in a number of tumor types are being performed (Raymond *et al*., 2000; Hidalgo *et al*., 2000). This review will summarize the principal mechanisms of anti-tumor action of rapamycin, specifically its effect on rapamycin-sensitive signal transduction pathways, and will discuss the preliminary results of experimental and clinical studies with this novel class of anti-cancer agents.

Mechanism of action of rapamcyin and rapamycin analogs

Rapamycin, and its ester analog, CCI-779, uniquely interfere with cell cycle progression from G_1 to S phase in response to proliferative stimuli by blocking the translation of mRNAs of essential cell cycle proteins (Wiederrecht *et al*., 1995). The principal mechanisms responsible for these actions, which have been elucidated only over the last several years, are graphically depicted in Figures 1 and 2.

Upstream actions and the target of rapamycin

Rapamycin binds intracellularly to members of the immunophilin family of FK506 binding proteins (FKBPs), inhibiting their enzymatic activity as prolyl isomerases (Heitman *et al*., 1991; Koltin *et al*., 1991; Fruman *et al*., 1995). Although there are many members of the FKBP family, a large body of biochemical and genetic studies suggest that FKBP12 is the most important binding protein with respect to the rapamycin-sensitive signal transduction pathway (Heitman *et al*., 1991; Koltin *et al*., 1991; Fruman *et al*., 1995). The resultant rapamycin-FKBP12 complex interacts with and inhibits the activity of a 290 kd kinase, termed mammalian target of rapamycin (mTOR) (also known as FRAP, RAFT1, and RAP1) (Figure 1) (Sabatini *et al*., 1994; Sabers *et al*., 1995; Brown *et al*., 1994; Chiu *et al*., 1994). mTOR is a member of a recently identified family of protein kinases termed phosphoinositide 3-kinase related kinases (PIKKs), which are involved in many critical regulatory cellular functions pertaining to cell cycle progression, cell cycle checkpoints that govern cellular responses to DNA damage, DNA repair, and DNA recombination (Sarkaria *et al*., 1998).

In response to growth stimuli, quiescent cells increase the translation of a subset of mRNAs whose protein products are required for traverse through the G_1 phase of the cell cycle. mTOR regulates essential signal transduction pathways and is involved in the coupling of growth stimuli with cell cycle progression. Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) (PI3K/Akt) appears to be the key modulatory factor in the upstream pathway by which growth factor-growth factor receptor interactions affect the phosphorylation state of mTOR (Figure 1) (Downward 1998; Scott *et al*., 1998; Nave *et al*., 1999). PI3K plays a central role in cellular proliferation, motility, neovascularization, viability, and senescence and is upregulated in cancer cells (Shayestech *et al*., 1999; Cantley *et al*., 1991). Its main physiological function is the phosphorylation of the D3 portion of membrane phosphoinositols (Cantley *et al*., 1991; Carpenter *et al*., 1990). Although the role of PI3K and its lipid products in signal transduction processes is not clear, the activity of this enzyme on tyrosine kinases induces mitogenesis, cellular growth, and cellular transformation (Carpenter *et al*., 1990; Varticovski *et al*., 1994; Hu *et al*., 1995). Recently, several studies have investigated the role of small molecule-inhibitors of PI3K as potential tumor suppressor agents. For example, the flavonoid derivative, LY294002 (Eli Lilly, Indianapolis, IN, USA), a potent PI3K inhibitor, is a competitive, reversible inhibitor of the ATP binding site of the enzyme (Vlahos *et al*., 1994; Hu *et al*., 2000). The agent induces G_1 arrest in proliferating cells, leading to almost complete inhibition of melanoma cell proliferation, partial inhibition of MG-63 osteosarcoma cell growth, and inhibitor of OVCAR-3 ovarian carcinoma inducing prominent apoptotic effects (Hu *et al*., 2000; Casagrande *et al*., 1998; Thomas *et al*., 1997). The inhibitor also completely inhibits the retinoblastoma protein (pRb) hyperphosphorylation that normally occurs during G1 progression and induces up-regulation of the cyclin-dependent kinase inhibitor p27 (Casagrande *et al*., 1998).

There are ample experimental data indicating that mTOR functions downstream of the PI3K/Akt pathway and is phosphorylated in responses to stimuli that activate the PI3K/Akt pathway (Scott *et al*., 1998; Nave *et al*., 1999; Hu *et al*., 1995; Sekulic *et al*., 2000). PI3K and Akt are considered proto-oncogenes, and the pathway is inhibited by the tumor suppressor gene *PTEN* (Wu *et al*., 1998). There are other signaling pathways that are activated downstream of PI3K, but the Akt pathway is of particular interest because of its role in inhibiting apoptosis and promoting cell proliferation by affecting the phosphorylation status

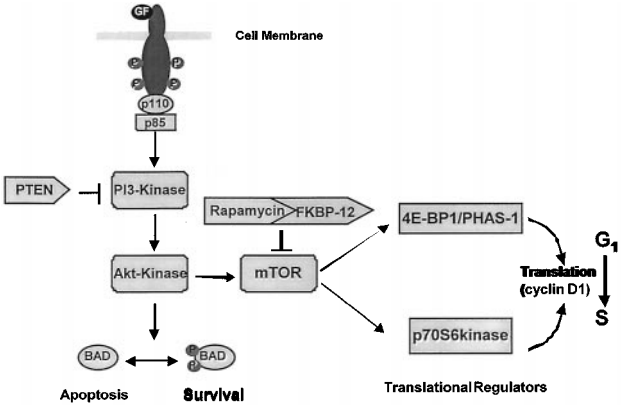

Figure 1 Rapamycin-sensitive signal transduction pathway. Rapamycin and CCI-779 bind to the immunophilin FK506 binding protein-12 (FKBP-12). The rapamycin-FKBP12 complex blocks the kinase activity of the mammalian target of rapamycin (mTOR). The inhibition of mTOR kinase activity inhibits the downstream translational regulators 4E-BP1/PHAS and $p70^{s6k}$. The inhibition of 4E-BP1/PHAS and $p70^{s6k}$ decrease the translation of mRNA of specific proteins essential for cell cycle progression from G1 to S phase

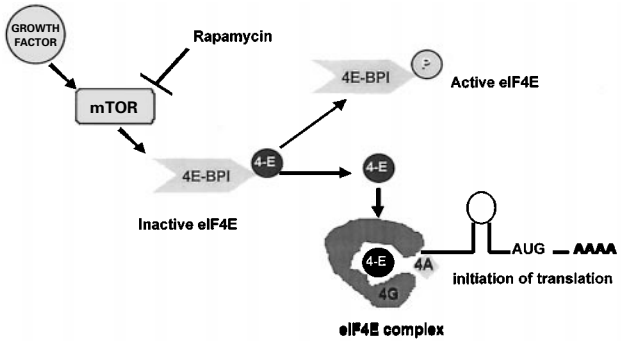

Figure 2 Rapamycin and CCI-779 inhibits the phosphorylation of 4E-BP1/PHAS, preventing the release of the eIF-4E and the activation of the eIF4F complex

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