mTOR as a Target for Cancer Therapy

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Abstract The target of rapamycin, mTOR, acts as a sensor for mitogenic stimuli, such as insulin-like growth factors and cellular nutritional status, regulating cellular growth and division. As many tumors are driven by autocrine or paracrine growth through the type-I insulin-like growth factor receptor, mTOR is potentially an attractive target for molecular-targeted treatment. Further, a rationale for anticipating tumor-selective activity based on transforming events frequently identified in malignant disease is becoming established.

1 Introduction

Since the early 1960s the introduction of cytotoxic agents, thence their dose intensification (starting in the 1970s), has dramatically improved survival of children with hematologic and solid tumors. For example, data from 1960–1963 shows that overall survival for children under fifteen years old with a diagnosis of neuroblastoma, bone or joint sarcomas, or CNS tumors was 25%, 20%, and 35%, respectively. In contrast, for the period 1985–1994 the survival for these same groups had increased to 63%–65%. However, for soft tissue sarcomas, the focus of this laboratory, increases in survival have been less impressive, increasing from 60% in 1974 to 71% (1985–1994)[1]. Although these results demonstrate clear progress in treating childhood malignancies, they do not reflect the morbidity and long-term sequelae often associated with intensive use of cytotoxic agents. Consequently, almost two decades ago we started to study pediatric soft tissue sarcomas with the ultimate goal of developing novel therapeutic approaches based on specific biological characteristics of these tumors. In this chapter we will review the process that allowed us to stumble onto rapamycin as a potential therapeutic agent, and the progress in understanding why this macrolide antibiotic may exert tumor-specific cytotoxicity.

2 Autocrine Growth of Rhabdomyosarcomas

Our laboratory has focused on rhabdomyosarcoma, a tumor of skeletal muscle origin, and in particular a particularly aggressive "alveolar" variant thereof. Cytogenetic analysis of several of these tumors from independent patients that were established as xenografts in immune-deprived mice showed a consistent chromosomal translocation t(2:13)(q35;q14; Hazelton et al. 1987). A more systematic survey of patient tumor biopsies demonstrated consistent translocations in greater than 90% of alveolar rhabdomyosarcomas (Douglass et al. 1987). We now know that this translocation results in expression of a chimeric tran-

[1] Years 1974 to present based on SEER data [Ries LAG, Miller BA, Guerney JG, Linet M, Tamra T, Young JL, Bunin GR (eds)]. SEER Program, 1975–1995; Tables and Graphs, National Cancer Institute. NIH Pub. No. 99–4649. Bethesda, MD, 1999

scription factor (PAX3/FKHR) that appears to block myogenic differentiation (Galili et al. 1993; Shapiro et al. 1993; Epstein et al. 1995) leading to tumor formation. These studies lead to further characterization of alveolar rhabdomyosarcoma cell lines, and revealed overexpression of transcripts for type II insulin-like growth factor (IGF-II) specifically from the fetal P3 promoter. In collaboration with Lee Helman at the Pediatric Branch, National Cancer Institute (NCI), Bethesda, we were able to show that growth of rhabdomyosarcoma cells was driven by an autocrine loop. Specifically, cells secreted IGF-II, and signaled through the IGF-I receptor (El-Badry et al. 1990). Inhibition of the IGF-I receptor by using a neutralizing antibody inhibited tumor cell growth. This suggested to us that interference with IGF-I-receptor-mediated signaling may be a therapeutic strategy for these tumors. To test this concept, the IGF-I receptor was downregulated using a stable expression of antisense constructs (Shapiro et al. 1994). These studies showed a high correlation between downregulation of the receptor, decreased growth in soft agar (such growth being a characteristic of malignant cells), and decreased formation of tumors when cells were inoculated into immune-deprived mice. Further, clones with the lowest expression of IGF-I receptor expressed the highest levels of the myogenic marker, MyoD, and formed multinucleate syncytia, thus recapitulating some characteristics of myogenic differentiation. While our studies focus on rhabdomyosarcomas, there is increasing evidence that many tumors are "driven" by either autocrine or paracrine signaling through the IGF-I receptor. Clearly deregulated IGF-I signaling is frequent in many pediatric solid tumors (neuroblastoma, Ewing's sarcoma, Wilms' tumor, medulloblastoma, glioblastoma), as well as many adult carcinomas (Macauly, 1992; Toretsky and Helman 1996). Direct inhibition of the IGF-I receptor with antibody, while effective in mouse models (Kalebic et al. 1994), was not at that time plausible in humans due to antigenicity, although this is now less of a problem and such reagents are being developed for clinical application. Alternatively, we sought a small molecule inhibitor of IGF-I signaling.

3
Selective Tumor Growth Inhibition by Rapamycin

Our studies with rapamycin started in 1992, and were stimulated by a chance conversation with Randall Johnson at SmithKline Beecham. At that time it was known that the immunosuppressive agents FK506 and

Table 1 Sensitivity of childhood rhabdomyosarcoma cell lines and human colon carcinoma cell lines to rapamycin and geldanamycin

	Rapamycin IC_{50} (ng/ml)	Geldanamycin C_{50} (nM)
Rhabdomyosarcoma cell lines		
Rh1	4,680	5.9
Rh18	0.1	14.3
Rh28	8.0	17.9
Rh30	0.37	1.9
Colon carcinoma cell lines		
GC_3/c1	9,800	3.6
VRC_5/c1	1,280	1.4
CaCo	1,570	3.4
HCT8	8,400	ND[a]
HCT29	>10,000	2.6
HCT116	>10,000	ND
National Cancer Institute screen (60 cell lines)	3,160	

[a] ND, not determined (from Dilling et al. 1994).

cyclosporin A blocked T cell activation prior to expression of interleukin-2, whereas rapamycin acted downstream of interleukin-2 expression (Flanagan et al. 1991; Schreiber and Crabtree 1992; McCaffrey et al. 1993). There was also some suggestion that for T cells to progress to S phase, the IGF-I receptor had to be expressed (Reiss et al. 1992). We speculated that perhaps rapamycin acted downstream of the IGF-I receptor to block cell cycle progression, and if so, may act to inhibit the growth of rhabdomyosarcoma cells. The results, shown in Table 1, were both surprising and exciting. Three of four rhabdomyosarcoma cell lines were exquisitely sensitive to rapamycin whereas one line, (Rh1), which is less dependent on IGF-I mitogenic signaling, was highly resistant. The other interesting aspect of these results was the marked selectivity for rhabdomyosarcoma cells relative to colon carcinoma cells, or cells used in the NCI in vitro screen (Dilling et al. 1994). Intriguing, but not comprehended (at that time) was the observation that under serum-free conditions Rh1 cells became very sensitive to rapamycin, with the IC50 decreasing from 5,800 ng/ml to 3.6 ng/ml. Consistent with results from other laboratories, the effect of rapamycin could be competed using FK506, indicating that initial formation of the FKBP-rapamycin complex was important.

4 Mechanism of Action of Rapamycin

The action of rapamycin will be covered in detail elsewhere. Briefly, rapamycin first binds the immunophilin FKBP-12 (the 12-kDa FK506 binding protein) and this complex is now known to be a specific inhibitor of a serine/threonine kinase mTOR (the mammalian target of rapamycin, also called FRAP/RAPT/RAFT; Brown et al. 1994; Sabatini et al. 1994; Chiu et al. 1994; Sabers et al. 1995). Kinase mTOR is a member of the PIKK superfamily that includes ATR, ATM, Mec1, and Tel1 proteins having homology to phosphatidylinositol lipid kinases. Evidence increasingly implicates mTOR as a central controller of cell growth and proliferation, and it controls initiation of translation of ribosomal proteins and several proteins that regulate cell cycle. Activation of ribosomal S6K1 after mitogen stimulation is dependent on mTOR (Chung et al. 1992; Kuo et al. 1992; Terada et al. 1992). Cap-dependent translation is facilitated by mTOR's phosphorylation and inactivation of 4E-BPs, suppressors of eukaryotic initiation factor 4E (eIF4E; Lin et al. 1994, Pause et al. 1994, Beretta et al. 1996). More recent findings have shown that mTOR may directly or indirectly control transcription, ribosomal biogenesis, actin cytoskeleton organization, and protein kinase C (reviewed in Schmelzle and Hall, 2000). Recently, our studies with rhabdomyosarcoma cells showed that activation of MAP kinases (p44/42) by growth factors was mTOR-dependent (Houghton et al. 2001; Harwood et al., manuscript submitted), implicating mTOR in cross-talk between the PI3K and MAPK pathways in some cell lines. Thus, the emerging picture places mTOR in a central role in which it senses mitogenic stimuli and amino acid (Iiboshi et al. 1999), ATP (Dennis et al. 2001), or nutrient (Rohde et al. 2001) conditions; mTOR coordinates many cellular processes related to growth and proliferation. The signaling pathways from IGF-IR to mTOR and downstream targets are depicted in Fig. 1.

5 Rapamycin Induces Apoptosis in Rhabdomyosarcoma Cells

The basis for the differential sensitivity of Rh1 cells under serum-containing or serum-free conditions was of interest. The results suggested that some component of serum was able to rescue Rh1 cells but not Rh30 cells. Consequently, we attempted to identify components of serum

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