Kinase Inhibitors in the Treatment of Canine Cancer  (18-Sep-2002)

C. A. London

University of California, Davis, Davis, CA, USA.

Protein kinases are enzymes that play key roles in cell signal transduction, regulating pathways critical in cell growth, differentiation, survival and death. These enzymes act by phosphorylating key residues on themselves and on other molecules, thereby generating a downstream signal inside the cell. The protein kinases are split into two major categories: tyrosine kinases (TKs) that phosphorylate proteins at tyrosine residues and serine/threonine kinases that phosphorylate proteins on serine and/or threonine. Some kinases are able to perform both functions. It is estimated that there are over 2000 serine/threonine kinases and 100 TKs encoded by the human genome. Those tyrosine kinases expressed on the cell surface that bind growth factor receptors are termed receptor tyrosine kinases (RTKs).

Role of Kinases in Tumor Cells
Dysfunction of many protein kinases, particularly the TKs, has been identified in a variety of human cancers, and is just beginning to be investigated in spontaneous tumors of dogs and cats. Such dysfunction can occur through mutation in the gene that encodes a kinase (point mutation, deletion, internal duplication, translocation, etc), or overexpression/amplification of the gene. In most cases, this dysfunction leads to inappropriate signaling of the kinase; i.e., the protein continuously signals without ever being turned "off". As a result, affected cells can begin to grow in an unregulated manner, contributing to the development of a malignant neoplasm. Examples of this include overexpression/amplification of HER2/neu in breast cancer, translocation resulting in a Bcr-Abl fusion protein in chronic myelogenous leukemia, point mutation in Ret in multiple endocrine neoplasia, missense mutation in Met in papillary renal cell carcinoma, and internal tandem duplications (ITDs) in Flt3 in acute myelogenous leukemia. The net effect of all of these mutations in essentially uncontrolled cell signaling. In veterinary medicine, ITDs have been identified in Kit in canine mast cell tumors, and overexpression of Met has been recognized in canine osteosarcoma. It is likely that more abnormalities will soon be characterized in spontaneous animal cancers.

Role of Kinases in Angiogenesis
While regulated kinase function is clearly important for normal cell function, it is also critical in the process of tumor angiogenesis, as most tumors cannot grow beyond a few millimeters in size unless they establish their own blood supply. Four RTKs have been identified as key players in this process: vascular endothelial growth factor receptor (VEGFR), platelet derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), and Tie1/2 (receptors for Angiopoietin). VEGFRs are expressed almost exclusively on vascular endothelium and VEGF-VEGFR interactions are probably critical for the induction of endothelial migration and proliferation. Both PDGF and PDGFR are expressed in stroma and pericytes (support cells for blood vessels), and PDGF has been shown to induce endothelial cell proliferation and migration in some studies. FGFR has been reported to be expressed on vascular endothelium, and FGF is synergistic with VEGF to induce the expression of VEGF. Lastly, both Tie1 and Tie2 are expressed on blood vessels found in several different types of human tumors. These receptors and their ligands are involved in the recruitment of pericytes and smooth muscle cells, as well as in maintaining vascular integrity. Recent evidence suggests that VEGFR and Tie signaling may act to coordinate the process of angiogenesis.

Development of Kinase Inhibitors
For the past several years, a great deal of effort has been directed at developing mechanisms to inhibit kinases that participate in the neoplastic process, both at the level of the cancer cell and the endothelial cell. A variety of approaches have been utilized and are described below.
1. Peptide inhibitors - Kinases within specific families use relatively conserved peptide sequences as substrates. That is, kinases recognize and activate downstream signaling molecules based on short sequences of amino acids. By slightly modifying the peptide sequence, the resultant peptide can act as a "pseudosubstrate," binding to the kinase and blocking the activation site, thereby preventing the true downstream molecule from binding. These peptide inhibitors often work very well in controlled biochemical reactions. However, many of these are incapable of entering intact cells and reaching their intended binding site. Therefore, while they have been of great use in studying the mechanism of action of kinases, they are probably not useful in a therapeutic setting.

2. Small molecule catalytic domain inhibitors - As previously described, kinases act to phosphorylate both themselves and other proteins to generate a downstream signaling cascade. For this to occur, the kinase must bind ATP to provide a phosphate group to the catalytic domain, which possesses the enzymatic activity of the protein. If ATP cannot be provided to the catalytic domain, the phosphorylation of other proteins will not occur. Given this fact, a large effort has been directed at blocking the ATP binding site of kinases to effectively shut off their activity. However, it would not be advantageous to block the kinase activity of all proteins, as many are critical for the function of normal, non-cancerous cells. To develop inhibitors that are more specific for particular kinases, investigators have characterized the ATP binding pockets of many receptors and found that the amino acid sequences in these pockets tend to be very well conserved among receptors within specific families. This knowledge has been used to construct competitive inhibitors that will only bind the pocket of a particular kinase family (such as the FGFRs). They often bind to the pocket more tightly than ATP, effectively preventing phosphorylation from taking place. Most of the small molecule inhibitors use a common core structure (chemical scaffold) that mimics the adenine moiety of ATP. Side chains are added to the molecule that can interact with other residues in the pocket. It is these side chains that drive specificity of binding, as they can be constructed to interact with particular amino acids found in the ATP pockets of a limited group of kinases. The small molecule inhibitors have an advantage over the peptide inhibitors as they often readily enter cells, thereby gaining access to their intended target.

3. Anti-receptor antibodies - Many of the kinases important in controlling tumor cell and endothelial cell growth are receptors for growth factors. Antibodies have been developed that target the extracellular domain of these receptors. This may act to prevent the intended growth factor from binding, or it may induce an immune response against the target cell. Another potential effect of antibody binding is the generation of an inappropriate downstream signal that may lead to target cell death.

4. SH2 domain inhibitors - SH2 domains are regions of cell signaling molecules that allow docking of one molecule to another. For example, the SH2 domain of Src, a well-described proto-oncogene, can bind phosphorylated tyrosines on receptor kinases. Once bound to the kinase, Src itself can be phosphorylated, thereby promoting the signaling cascade. Both peptide mimics and non-peptide mimics have been designed that will bind to SH2 domains of molecules like Src, serving to interrupt this cascade.

Kinase inhibitors in the treatment of canine cancer

Small molecule inhibitors termed indolinones were developed to competitively inhibit the ATP binding site of a variety of RTKs. Phase I clinical trial of a novel orally administered multi-targeted indolinone kinase inhibitor, SU11654 was conducted in dogs with spontaneous tumors. SU11654 acts as competitive inhibitor of ATP binding to several RTKs known to be important in angiogenesis and tumor cell growth including VEGFR, FGFR, PDGFR, Flt3, and Kit. Fifty-seven dogs diagnosed with recurrent or metastatic tumors were enrolled into a dose escalating study beginning in March 2001. Of those 57 dogs, 47 finished the initial 3-week cycle and were considered assessable for response (remaining dogs typically experienced rapidly progressive disease). Overall, signs of biological activity (tumor regression or stable disease > 10 weeks) were observed in 68% (n=32) of dogs. Mast cell tumor (MCT) was the most common tumor type (n=22). Of these, 11 MCTs possessed an activating Kit mutation; the biological activity (3CR + 6PR + 1SD) was 91% (n=10) in this group. Biological activity (1 CR, 1 PR, 1 SD) in MCT with no Kit mutation was lower (27%), suggesting that tumors with Kit mutation are more responsive to SU11654 therapy. Objective tumor regressions (PR/CR) were also observed in mammary carcinoma (2 of 5 dogs, with the remaining 3 dogs having SD at 13, 14+ and 36+ weeks); soft tissue sarcoma (2 of 3 dogs); and multiple myeloma (n=1). Toxicities were typically mild and included neutropenia, occasional hind limb weakness, diarrhea (mild-to-moderate in severity) responsive to metronidazole therapy, and anorexia responsive to brief cessation of dosing. In summary, SU11654 exhibited activity against a variety of spontaneous tumors in dogs suggesting that it may have a role in the future treatment of such neoplasms.
Biographical Profile

After graduating from Tufts School of Veterinary Medicine in 1990, Dr. Cheryl London worked in private practice for 2 years in Maine. She discovered a great interest in cancer, and subsequently completed a residency in Medical Oncology at the University of Wisconsin, Madison. During that time, she found she truly enjoyed performing cancer research in the laboratory, and when her residency was completed in 1994, she entered a Ph.D. program in Immunology at Harvard University. Dr. London graduated in 1999, and have since been an Assistant Professor of Medical Oncology at UC Davis. Her research interests center primarily around the use of kinase inhibitors in cancer therapy.

All rights reserved. This document is available on-line at www.ivis.org. Document No. P0411.0902.