The Phosphoinositide 3-Kinase Pathway Is Crucial for the Growth of Canine Mast Cell Tumors

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ABSTRACT. Mast cell tumors (MCTs) are the most common tumors in dogs, accounting for 16–21% of cutaneous tumors. Although several small molecule inhibitors, including imatinib mesylate, have been used for the treatment of MCTs, the response rate remains less than 50%. In this study, the effects of different selective signal inhibitors on MCT cell growth were evaluated using 4 different cell lines derived from dogs. We found that the phosphoinositide 3-kinase (PI3K) signaling pathway is crucial for the proliferation of MCT cells in the presence or absence of c-kit gene mutations. Here, we propose a novel therapeutic strategy to target the PI3K pathway for the treatment of canine MCTs.

KEY WORDS: mast cell tumor, PI3K signaling.

Mast cell tumors (MCTs) are one of the common malignant tumors in dogs and are characterized by the aberrant expansion of mast cells [30]. MCTs often relapse or behave in an aggressive manner, metastasizing to local lymph nodes, the liver and the spleen [30]. Chemotherapeutic agents, such as vinblastine, cyclophosphamide and prednisone are used to treat MCTs [6, 10]. Among these, prednisone has yielded the most favorable results leading to 50% reduction in tumor size of more than 75% of MCTs [17]. Because long-term use of prednisone causes kidney or liver dysfunction and iatrogenic Cushing disease [12], combination chemotherapy with additional selective molecular inhibitors and a low dose of prednisone may exert potent antitumor activity with minimal adverse side effects. The small molecule inhibitors, toceranib phosphate and masitinib mesylate target KIT activation. In recent years, the use of these inhibitors for the treatment of dog MCTs has been approved by the Food and Drug Administration and European Medicines Agency [9, 15]. Interestingly, approximately 50% of MCTs, including KIT mutation-negative tumors, were found to be sensitive to masitinib mesylate [9]. In addition, toceranib phosphate also achieved a complete or partial response in 42.8% of MCTs without juxtamembrane domain mutations [15], which is one of the most crucial mutations for tumor formation [14, 31]. However, it is evident that the targeting KIT strategy will be effective for no more than 50% of MCTs, and the remaining cases will require other protocols. Therefore, it is necessary to identify therapeutic targets that can broadly inhibit the growth of neoplastic mast cells.

Activation of multiple signaling pathways, such as the Janus kinase/signal transducer and activator of transcription (JAK/STAT), mitogen-activated protein kinase (MAPK) or phosphoinositide 3-kinase (PI3K) pathway, plays important roles in the proliferation and differentiation of hematopoietic cells, such as leukemia cells [5, 22, 26, 29, 32]. In mast cells, these signaling pathways are also essential for their growth, survival and proper cell function [8, 13, 16, 19, 25]. However, the signaling pathways required for the growth of neoplastic mast cells, especially for canine MCT cells, remain unknown. In this study, we evaluated the effects of several selective inhibitors of cell proliferation on canine MCT lines to identify the major signaling pathway involved in canine mast cell growth.

We used 4 MCT cell lines that express different types of wild-type or mutant KIT proteins. These 4 lines were HRMC cells that express wild-type KIT [21], BR cells that express KIT with a point mutation in the juxtamembrane domain [7], MPT-2 cells that express KIT with an internal tandem duplication in the juxtamembrane domain [17] and MPT-1.2 cells, a newly-established subline of MPT-1 cells that express KIT with an N508I point mutation in the extracellular domain [2]. Except for the BR cell line, the remaining 3 cell lines were maintained in RPMI 1640 medium (Life Technologies, Gaithersburg, MD, U.S.A.) supplemented with 10% FBS (Hyclone, Logan, UT, U.S.A.) and antibiotics. BR cells were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies) supplemented with 10% FBS and antibiotics. In addition to the KIT inhibitor STI571 (also known as ima...
Fig. 1. Effect of each inhibitor on the proliferation of neoplastic mast cells. The absorbance of each cell line that was incubated with either STI571 (A), LY294002 (B), rapamycin (C), AG490 (D) or PD98059 (E). The experiment was conducted 3 times, and the mean ± SD values are shown. ** P<0.01 as compared to the control by Williams’ test.
As shown in Fig. 1, the growth of mutant KIT-expressing 3 cell lines (BR, MPT-1.2 and MPT-2) was suppressed by STI571, but wild-type KIT-expressing HRMC cells were not affected (Table 1, Fig. 1A). Interestingly, the PI3K signaling inhibitors, both LY294002 and rapamycin, reduced the growth of all MCT cell lines regardless of the type of KIT protein expressed (Fig. 1B and 1C). Particularly, rapamycin showed potent inhibitory effects on cell proliferation of all cell lines with the lowest IC50 values (Table 1). Inhibitors of either JAK/STAT or MAPK signaling had no or little effect on growth (Fig. 1D and 1E). However, the IC50 values of AG490 or PD98059 were barely determined in MPT-2 cells, as the much higher concentration was required to suppress PI3K signaling pathway may be a viable target to treat canine MCTs. In general, combination therapies are superior to monotherapy for the better clinical outcome, probably because they can reduce the resistance to antitumor agents. For example, everolimus in combination with tamoxifen improves the survival rate of patients with aromatase inhibitor-resistant breast cancer [3]. Thus, combining PI3K inhibitors with other agents, such as glucocorticoid, may be the alternative strategy for the better remission of KIT inhibitor-resistant MCTs.

In conclusion, the PI3K signaling pathway may be a key regulator for the growth of MCT cells regardless of the type of KIT protein expressed. Given the necessity of PI3K signaling pathway in neoplastic mast cell proliferation, we strongly suggest that targeting PI3K signaling would be a novel therapeutic approach to treat canine MCTs.

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