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Short Communication

Identification of dasatinib as an in vitro potent growth inhibitor of canine histiocytic sarcoma cells

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ABSTRACT

Canine histiocytic sarcoma (HS) is an aggressive and fatal neoplasm that has a high recurrence rate and metastatic nature. In the present report, compounds were screened for their growth inhibitory activity in two HS cell lines using a chemical library known to target specific signalling pathways. Among 171 compounds screened, dasatinib, which targets several types of kinases, clearly inhibited cell growth in one of the two HS lines. The growth inhibitory properties of dasatinib were then examined using six HS cell lines and MDCK cells. Dasatinib demonstrated potent growth inhibitory activity against four HS cell lines with calculated IC50 values of 5.4–54.5 nM, while the IC50 values in the other cell lines were in the micromolar range. In conclusion, a kinase enzyme targeted by dasatinib appears to be crucial for growth in some subsets of HS and the on-target activity of dasatinib could underlie the marked growth inhibition in HS cells.

Canine histiocytic sarcoma (HS) is an aggressive neoplasm that originates from a histiocytic lineage including macrophages and dendritic cells (Moore et al., 2006). Due to the aggressive nature of HS, chemotherapeutic agents such as *N*-(2-chloroethyl)-*N*′-cyclohexyl-*N*-nitrosourea (CCNU) have often been used for the treatment of HS, but canine HS is usually fatal with short survival (Skorupski et al., 2007). Therefore, a new therapeutic approach is required for the treatment of HS in dogs.

Targeted therapy using a compound that blocks the growth of malignant cells by interfering with specific targeted molecules needed for tumorigenesis and tumour growth is a potent therapeutic approach for malignancies and has been shown to be effective in the treatment of many types of malignant cancer in humans (Keefe and Bateman, 2011) as well as mast cell tumours in dogs and cats (London, 2009). Despite the promising strategy for the treatment of malignancies, no compound for targeted therapy against canine HS has been identified. Here, using a chemical library consisting of 171 compounds that are clinically used or known to target specific signalling pathways, we screened for compounds that have growth inhibitory potency in canine HS cells.

Previously established HS cell lines (CHS-1, CHS-2, CHS-4, CHS-5, and CHS-7) (Azakami et al., 2006) and a newly established HS cell line, MHT-2, were maintained in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum (Nippon Bio-supply) (cDMEM). For the screening of the compounds that have growth inhibitory effect in HS cells, two HS lines (CHS-1 and MHT-2) and 171 inhibitors (Table 1) from the Screening Committee of Anticancer Drugs (Japan) were used. The HS cells suspended in cDMEM were placed in 96-well plates (5×10^3 cells/well) and incubated for 24 h. The medium was then replaced with cDMEM containing 0.1 μM of each chemical compound and cultured for 72 h. After culture, cell viability was evaluated using a WST-1 cell proliferation assay kit (Takara) and compounds that showed more than 60% inhibition of cell growth were identified.

The effects of compounds on the viability of CHS-1 and MHT-2 cells are shown in Fig. 1. Among 171 compounds, eight (12, 58, 66, 77, 86, 87, 116 and 153) suppressed cell growth more than 60% in CHS-1 and/or MHT-2 cells. These compounds were then examined in the same manner for any growth inhibitory effect on non-HS canine cells, i.e. MDCK (Madin-Darby canine kidney) cells. Other than compound 77 (dasatinib) the compounds showed a growth inhibitory effect in MDCK cells (data not shown), suggesting that all except dasatinib have some non-specific cytotoxic effect at this concentration. Although dasatinib slightly suppressed the growth of MHT-2 cells, it clearly inhibited the growth of CHS-1 cells (Fig. 1).

We then focused on dasatinib and examined its growth inhibitory property against six HS lines with MDCK cells used as a control. The cell lines were cultured in 96-well plates (HS cells, 5×10^3 cells/well; MDCK cells, 5×10^2 cells/well) for 24 h in cDMEM and then treated with different concentrations of dasatinib (LC Laboratories) (0–10^5 nM) for 72 h. Cell viability was measured

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Table 1
Compound characteristics.

No.	Category Target	Compound name
	Receptor kinase	
1	EGFR	AG1478
2	HER2	AG825
3	EGFR, topoII	Genistein
4	EGFR	BPIQ-II
5	EGFR	AG490
6	EGFR/Her2	Lapatinib
7	EGFR	Gefitinib
8	EGFR	Erlotinib
9	PDGFR	AG1296
10	PDGFR	SU11652
11	PDGFR	PDGF receptor tyrosine kinase inhibitor V
12	PDGFR	PDGF receptor tyrosine kinase inhibitor IV
13	VEGFR	VEGFR receptor tyrosine kinase inhibitor II
14	VEGFR	VEGF recptor 2 kinase inhibitor I
15	VEGFR	SU1498
16	FGFR	SU4984
17	FGFR	SU5402
18	IGF-IR	AG1024
19	IGF-IR	AGL2263
20	TrKA	TrkA inhibitor
21	Flt-3	Flt-3 inhibitor
22	Fms	cFMS receptor tyrosine kinase inhibitor
23	Met	SU11274
24	TGF-βRI	SB431542
25	TGF-βRI	TGF-β RI kinase inhibitor II
	Fusion kinase	
26	Bcr-abl	AG957
27	Bcr-Abl, Kit, PDGFR	Nilotinib
28	Bcr-Abl, Kit, PDGFR	Imatinib mesylate
29	EML4-ALK	Crizotinib
	Multiple kinase	
30	Multi-kinases	Sorafenib
31	Multi-kinases	Sunitinib malate
32	Multi-kinases	Pazopanib
	Chemokine receptor	
33	CCR2	RS102895
34	CCR3	SB328437
35	CXCR2	SB225002
36	CXCR4	AMD3100 octahydrochloride
	PI3K-AKT pathway	
37	PI3K	LY-294002
38	PI3K	Wortmannin
39	AKT	AKT inhibitor
40	AKT	NL-71-101
41	AKT	Akt inhibitor IV
42	AKT	Akt inhibitor VIII, isozyme-selective, Akti-1/2
43	AKT	Akt inhibitor XI
	MAPK pathway	
44	Raf	RAF1 kinase inhibitor I
45	Raf	ZM336372
46	MEK	PD98059
47	MEK	U-0126
48	MEK	MEK inhibitor I
49	Tpl2	Tpl2 kinase inhibitor
50	MAPK	ERK inhibitor II
51	JNK	SP600125
52	JNK	JNK inhibitor VIII
53	p38 (MAPK)	PD169316
54	p38 (MAPK)	SB203580
55	p38	SB202190
56	p38	SB239063
	JAK-STAT pathway	
57	Jak-2	AG490
58	Jak-2	Cucurbitacin I
59	Jak	JAK inhibitor I
60	Jak	JAK3 inhibitor VI
61	STAT3	WP1066
62	STAT3	5,15-DPP
	mTOR pathway	
63	mTOR	Temsirolimus
64	mTOR	Everolimus
65	mTOR	Torkinib
66	p70 S6K	Rapamycin
67	eEF2	TX-1918
	Rho-ROCK pathway	
68	Rho/SRF	CCG-1423
69	ROCK	HA1077
70	ROCK	H-1152
71	ROCK	Y-27632
	SRC family kinase	
72	Lck	Damnacanthal
73	Src	PP1 analog
74	Src, Fyn, Lck	PP-H
75	Fyn, Yes, Src, Lyn	SU6656
76	Lck, Fyn	PP2
77	Bcr-Abl, Src	Dasatinib
	Glycogen synthase kinase	
78	GSK	GSK-3 inhibitor IX
79	GSK	1-azakenpaullone
80	GSK	Indirubin-3′-monoxime
81	GSK-3	GSK-3 inhibitor II
	Burton's tyrosine kinase	
82	BTK	LFM-A13
83	BTK	Terreic acid
	Spleen tyrosine kinase	
84	Syk	Syk inhibitor
	IL-1 receptor-associated kinase	
85	IRAK	IRAK-1/4 inhibitor
	Heat shock protein	
86	HSP90	Radicicol
87	HSP90	17-AAG
	Cyclooxygenase	
88	COX-1	Sulindac sulfide
89	COX-1	Valeryl salicylate
90	COX-2	NS-398
91	COX	Sodium salicylate
	Nitric oxide synthase	
92	iNOS	1400W, HCl
93	iNOS	AMT, HCl
94	NOS	Aminoguanidine, HCl
95	NOS	L-NMMA
	Protein phosphatase	
96	PP2A	Cantharidin
97	PP2A	Cytostatin
98	PP2B/cyclophilin	Cyclosporin A
99	PP2B/FKBP	FK-506
	Cell cycle related molecule	
100	CDC2	Kenpaullone
101	Cdc25	NSC95397
102	Cdc25A	SC-ααασ9
103	CDK2	Purvalanol A
104	CDK4	3-ATA
105	CDKs	Olomoucine
106	CDK	Kenpaullone
107	CDK	Purvalanol A
108	CDK	Olomoucine
109	CDK	Alsterpaullone, 2-cyanoethyl
110	CDK	Cdk1/2 inhibitor III
111	CDK	Cdk2/9 inhibitor
112	CDK	NU6102
113	CDK	Cdk4 inhibitor
114	CDK	NSC625987
	Chromatin/chromosome modification	
115	HDAC	Scriptaid
116	HDAC	Trichostatin A
117	HDAC	Vorinostat
118	HAT	Anacardic acid
119	Telomerase	MST-312
120	Telomerase	β-rubromycin
	Mitosis related molecule	
121	Aurora	Aurora kinase/cdk inhibitor
122	Aurora	Aurora kinase inhibitor II

(*continued on next page*)