Aurora Kinase A Is Upregulated in Cutaneous T-Cell Lymphoma and Represents a Potential Therapeutic Target

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Cutaneous T-cell lymphomas (CTCLs) form a heterogeneous group of non-Hodgkin's lymphomas characterized by only poor prognosis in advanced stage. Despite significant progress made in the identification of novel genes and pathways involved in the pathogenesis of cutaneous lymphoma, the therapeutic value of these findings has still to be proven. Here, we demonstrate by gene expression arrays that Aurora kinase A is one of the highly overexpressed genes of the serine/threonine kinase in CTCL. The finding was confirmed by quantitative reverse transcriptase–PCR, western blotting, and immunohistochemistry in CTCL cell lines and primary patient samples. Moreover, treatment with a specific Aurora kinase A inhibitor blocks cell proliferation by inducing cell cycle arrest in G2 phase, as well as apoptosis in CTCL cell lines. These data provide a promising rationale for using Aurora kinase A inhibition as a therapeutic modality of CTCL.

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INTRODUCTION

Cutaneous T-cell lymphomas (CTCL) represent a heterogeneous group of extranodal T-cell lymphomas that by definition originate through clonal expansion of neoplastic T cells homing to the skin without further systemic involvement at the time of diagnosis. Mycosis fungoides (MF) with an incidence of 4–5/1,000,000 inhabitants per year is the most common form of CTCL, accounting for ~50% of CTCLs (Criscione and Weinstock, 2007). Three clinical stages are distinguished in disease progression: initial erythematous macules (patch stage) are often followed by deeper infiltrated plaques (plaque stage), and in some cases skin tumors may develop (tumor stage). On the other hand, Sézary syndrome (SS), an aggressive variant of primary CTCL, is characterized by neoplastic T cells in skin, lymph nodes, and peripheral blood already from the beginning of the disease (Willemze *et al.*, 2005).

At present, there is only limited understanding on the pathogenesis of CTCL. Cytogenetic studies and gene expression analyses of the past years could identify several genes that may have a major role in the development and/or progression of CTCL. The so far identified genes enclose several known oncogenes and tumor suppressor genes that are involved in major pathways regulating the cell cycle, cell survival, or apoptosis—e.g., *MYC*, *TP53*, *NOTCH1*, *E2A*, and *CDKN2A* (p16), and *CDKN2B* (p15) (Vermeer *et al.*, 2008; Kamstrup *et al.*, 2010; Marks *et al.*, 1996; Steininger *et al.*, 2011; Lamprecht *et al.*, 2012; Manfé *et al.*, 2012). These changes were particularly identified in tumor- or plaque-stage MF lesions, as well as in circulating tumor cells of SS patients, owing to the high numbers of tumor cells in these situations. It remains therefore an open question of whether these genes are also altered in early stages and may thus contribute to genesis or progression of CTCL.

Despite significant progress in the identification of genes and pathways involved in pathogenesis of CTCL, the therapeutic value of these findings is still low, and there remains a particular need for treatments of patients with advanced-stage CTCL.

The three Aurora kinase (AURK) family members (A, B, and C) represent serine/threonine kinases and are key regulators of mitosis as well as diverse signal transduction pathways for control of, e.g., centrosome function, mitotic entry, kinetochore function, spindle assembly, chromosome segregation,

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Abbreviations: AURK, Aurora kinase; AURKA, Aurora kinase A; cDNA, complementary DNA; CDK, cyclin-dependent kinase; CTCL, cutaneous T-cell lymphoma; MF, mycosis fungoides; PLK1, polo-like kinase 1; SS, Sézary syndrome

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Table 1. Top 20 upregulated serine/threonine kinases in Mycosis fungoides versus normal skin

Gene symbol	Patch/plaque (PP) versus normal		Tumor (Tu) versus normal		PP versus Tu		Description
	FCH	FDR	FCH	FDR	FCH	FDR	
PIK3CG	7.70	0.00024	13.18	0.00019	1.71	0.64	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit-γ
MAP4K1	4.11	0.0038	11.92	0.00018	2.90	0.20	Mitogen-activated protein kinase kinase kinase kinase 1, HPK1
BUB1	2.36	0.22	7.69	0.018	3.26	0.39	BUB1 mitotic checkpoint serine/threonine kinase
STK17A	4.51	0.0013	7.07	0.00085	1.57	0.68	Serine/threonine kinase 17a
STK17B	4.73	0.000303	7.04	0.00025	1.49	0.67	Serine/threonine kinase 17b
AURKA	3.56	0.048	6.94	0.016	1.95	0.67	Aurora kinase A
LIMK1	2.38	0.00074	6.14	2.98E-06	2.58	0.021	LIM domain kinase 1
PRKCB	2.02	0.039	6.07	0.00018	3.01	0.058	Protein kinase C, β
PIM2	3.50	0.00030	5.94	7.26E-05	1.70	0.38	Pim-2 oncogene
PBK	1.78	0.32	5.61	0.014	3.16	0.27	PDZ-binding kinase
PLK4	1.61	0.19	3.21	0.011	1.99	0.32	Polo-like kinase 4
STK4	3.91	0.00044	4.57	0.0010	1.17	0.88	Serine/threonine kinase 4
AURKB	1.27	0.57	4.21	0.0040	3.31	0.072	Aurora kinase B
NEK6	1.64	0.092	4.15	0.00044	2.53	0.065	NIMA-related kinase 6
TGFBR1	2.01	0.042	4.11	0.0018	2.05	0.26	Transforming growth factor, β receptor 1
CHEK1	1.96	0.16	4.07	0.019	2.07	0.47	Checkpoint kinase 1
BUB1B	1.16	0.76	3.81	0.011	3.29	0.097	BUB1 mitotic checkpoint serine/threonine kinase B
BMP2K	2.11	0.0058	3.78	0.00028	1.79	0.22	BMP2 inducible kinase
MST4	1.19	0.63	3.29	0.0050	2.75	0.075	Serine/threonine protein kinase MST4
NEK2	2.24	0.23	3.36	0.14	1.5	0.84	NIMA-related kinase 2

Abbreviations: FCH, fold change; FDR, false discovery rate.

cytokinesis, and interaction with p53, p73, and cMYC. Overexpression of particularly Aurora kinase A (AURKA) has been linked to tumorigenesis and has already been demonstrated in several hematologic malignancies as well as in solid tumors. This resulted in the development of several AURK inhibitors that are currently being tested in first clinical trials and have become a promising therapeutic option in cancer therapy.

In an approach to further define pathways and to identify novel potential targets for therapeutic intervention, we have investigated gene expression profiles of CTCL samples of different stages compared with normal skin by microarray and real-time reverse transcriptase–PCR analysis. A number of genes involved in regulation of the cell cycle and mitosis were found to be upregulated in CTCLs. Among these, AURKA revealed a most significant upregulation in MF skin samples as compared with normal skin. Overexpression of AURKA in CTCL was also confirmed by real-time reverse transcriptase–PCR analysis of circulating tumor cells of SS patients and in CTCL cell lines. Interestingly, inhibition of AURKA in CTCL cell lines with MLN8237, a specific AURKA inhibitor, results in a massive reduction of viable cells already after 24 hours. This reduction was caused by cell cycle arrest in G2/M phase and in addition by induction of apoptosis in tested cell lines, indicating that AURKA inhibition could represent a promising therapeutic strategy for CTCL patients.

RESULTS

Genes involved in cell cycle control and mitosis are significantly upregulated in CTCL

To identify pathways that are activated in CTCL, we performed gene expression profiling of 13 lesional skin biopsies of different MF patients (patch = 3, plaque = 6, tumor = 4) in comparison with healthy control tissue from 8 patients. Using criteria of >2.0-fold change and false discovery rate of <0.05, we identified a series of differentially expressed genes. Our initial analysis focused on genes encoding serine/threonine kinases and serine/threonine/tyrosine receptor kinases, corresponding to Gene Ontology terms GO:0004674 and GO:0004712, respectively. Within this category, we found AURKA as one of the 10 most upregulated genes (Table 1). Based on these findings that AURKA was one of the highest serine/threonine/tyrosin receptor kinase expressed, and in addition the information that a highly specific inhibitory agent for potential treatment is already available, we focused our further descriptive and functional analyses on AURKA.