CHAPTER 10

SRC KINASE IN UVEAL MELANOMA IS ASSOCIATED WITH MONOSOMY 3 AND CAN BE INHIBITED BY DASATINIB

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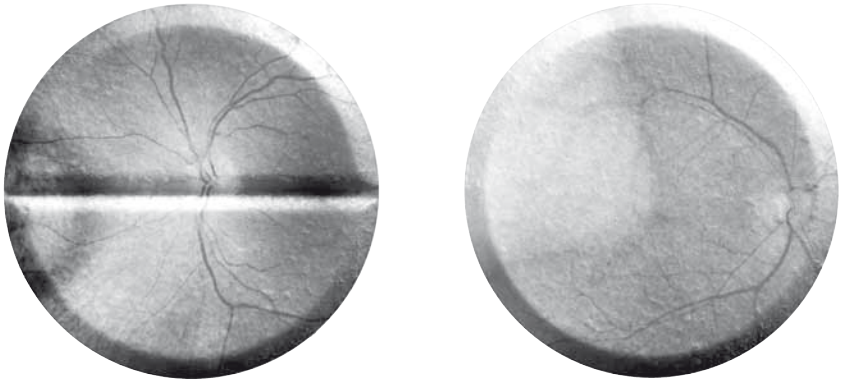

ABSTRACT

Purpose: Uveal melanoma (UM) leads to metastasis in up to 50% of the patients. Patients at risk are readily identified using an array of prognostic markers but an effective treatment is lacking. We recently identified Src as an important tyrosine kinase that conveys MAPK activation in UM. GNAQ/GNA11 mutations are the common denominators of oncogene signalling in UM and Src is a likely downstream kinase. We describe the preclinical analysis of Dasatinib, a known inhibitor of Src kinase, in UM.

Experimental design: Eight tumours were removed, cultured and exposed to Dasatinib treatment. Proliferation and MAPK signalling were evaluated. Additionally, 36 UM samples were analyzed for Src/ERK signalling, GNAQ/GNA11 mutation status, chromosome 3 and known histological prognostic parameters.

Results: Growth arrest was observed in 5 of 8 UM cultures and molecular analysis indicated that Dasatinib inhibited MAPK via Src. Treatment efficacy associated with MAPK and Src kinase activity as UM cells with the highest Src activity and MAPK activation displayed the strongest growth inhibition. Furthermore, treatment responses tended to be better in UM cultures with monosomy 3. In the cohort of primary UM, Src expression was highly correlated with MAPK activation and monosomy 3.

Conclusions: We identified the Src family kinase inhibitor, Dasatinib, as a treatment option for UM. Dasatinib inhibits UM proliferation and may also inhibit UM progression as an association between Src and monosomy 3 was revealed. Consequently, monosomy 3 analysis in tumour tissue may suffice both the prognosis and choice of treatment.

GENERAL INTRODUCTION

Uveal melanoma (UM) is a rare tumour affecting 7/1.0 million of the Western population per year [1]. Still, it is the most common tumour in the eye in adults; 30 % of the tumours is asymptomatic and only discovered at routine examination [2]. This malignancy may ultimately lead to metastatic disease in up to 50% of the patients [3].

UM commonly spreads hematogeneously because there are hardly any lymph vessels present in the eye, and most often metastasis of UM are found in the liver. Once liver metastases have been detected, life expectancy is a mere 2-6 months on average since hardly any effective treatment is available for these patients [4]. This highlights the necessity for effective adjuvant and clinical treatment options which can be applied in high risk and metastasis patients.

Identifying high risk patients is possible with an array of diagnostic and prognostic markers. Well-known prognostic factors for UM are an advanced age, tumour thickness/diameter, and localization in the ciliary body [5]. Besides these histopathological parameters, there are also genetic aberrations that are associated with a poor prognosis. Most significantly, loss of one copy of chromosome 3 is observed in almost half of the tumours and is associated with a poor prognosis [6]. The risk associated with monosomy 3 is further modulated by other chromosomal aberrations such as deletion of chromosome 1p and gain of chromosomes 6p and 8q [7]. How these aberrations, besides being a prognostic factor, contribute to the metastasis of UM is still unknown but the recent detection of truncating mutations in the BAP1 gene may elucidate a possible mechanism [8].

Recently, two recurrent mutations were identified in the GNAQ and the GNA11 genes. For both the GNAQ and the GNA11 gene, a mutation in glutamine-encoding codon 209 has been described (Q209), as well as a mutation in R183 (less frequent). Mutations in the GNAQ and GNA11 gene were shown to be common in UM with frequencies of respectively 45% and 32% [9,10]. GNAQ and GNA11 encode for Gαq-type subunits of the heterotrimeric G-protein and the mutations result in constitutive G-protein activation which mediates intracellular signals and activates the Mitogen-activated protein kinase (MAPK) pathway, promoting proliferation and cell survival [11]. Cellular components that couple GNAQ/GNA11 to MAPK signalling are most likely the effector IP3 and Src tyrosine kinase [12].

Recently, we showed that in UM, Src expression and Src kinase activity is correlated with MAPK activation [13]. Whether Src is the intermediate kinase between oncogenic GNAQ/GNA11 signalling and MAPK activation in UM remains to be shown, but it potentially provides an opportunity for pharmaceutical intervention. Treatment of UM cell lines with specific MAPK and Src inhibitors has already been shown to reduce proliferation [13], demonstrating the importance of the MAPK pathway and Src kinase for UM growth.

Dasatinib is an inhibitor of the Src-family kinases as well as other kinases such as BCR-ABL, c-Kit, PDGFR-alpha and beta, and ephrin-receptor kinase [14,15]. It has been approved for the treatment of patients with BCR-ABL-positive chronic myeloid leukaemia (CML),

Philadelphia Chromosome positive (Ph+) acute lymphoblastic leukaemia (ALL) and results in remission and improved survival in these malignancies. For several other diseases including solid tumours, Dasatinib is still under investigation [16]. The single daily oral dose of 100 mg, and manageable side effects, make Dasatinib an interesting candidate for treatment of patients with metastatic disease and/or patients with high risk UM.

We investigated in vitro the efficacy of Dasatinib and analyzed the underlying molecular mechanisms in order to identify potential biomarkers.

MATERIALS AND METHODS

Patient tumour material and primary cell cultures

Informed consent was obtained prior to enucleation and the study was performed according to the tenets of the Declaration of Helsinki. Diagnosis was made based on histopathology of the tumor samples. Fresh tumor tissue (n=36), obtained immediately after enucleation of the eye, was frozen in liquid nitrogen-cooled isopentane and used to isolate DNA, protein lysates or to develop a cell culture (n=8). Histologic sections were prepared from tissues fixed in 10% neutral-buffered formalin for 48 hours and embedded in paraffin. Hematoxylin-eosin-stained 4-µm sections were examined for cell type, largest basal diameter, prominence, ciliary body involvement and scleral invasion.

Primary tumour cell cultures

Primary UM cell cultures (n=8) were cultured in Amniochrome® Pro Medium (Lonza Group Ltd, Basel, Switzerland) and incubated in an atmosphere with 5% CO_2 at a temperature of 370C. (HERAcell 240 CO_2 Incubator, Thermo Fisher Scientific Inc, USA). The cells were passaged once or twice a week using trypsin (0.05%).

PCR

GNAQ/GNA11 genes were amplified with a Polymerase Chain Reaction (PCR). A PCR was conducted using chromosomal DNA, isolated from frozen tumours using the QIAamp DNA minikit from Qiagen (Germany). The following protocol was used for amplification of exon 5 of the GNA11 and the GNAQ genes:

94°C, 1min; (96°C, 15sec; 63°C, 15sec; 72°C, 1min) 7X; (96°C, 15sec; 61°C, 15sec; 71°C, 1min) 8X; (96°C, 15sec; 60°C, 15sec; 72°C, 1min), 36X; 72°C, 1min, end.

The following primers were used CGCTGTGTCCTTTCAGGATGGTG, GNA11 Forward and GCCCACCTCGTTGTCCGACT, GNA11 Reverse.

The forward primer for GNAQ: CCCTAAGTTTGTAAGTAGTGCTATATTTATGTTG,

while the reverse primer was ATGATAATCCATTGCCTGTCTAAAGAACAC. After amplification, DNA clean-up was performed using the Nucleospin Extract II columns of Macherey-Nagel (Germany) following the manufacturers protocol. For sequencing analysis, samples were prepared by adding 10 pmol of reverse primer to the amplified DNA.

Sequencing and Karyotyping

Sequencing for GNAQ c.626A>C (~30%) and GNAQ c.626A>T (~70%) and GNA11 c.626A>T (100%) mutations was performed at the Leiden Genomic Technology Center (LGTC) department of the LUMC. Screening for monosomy 3 with karyotyping in all uveal melanoma samples and primary cultures was performed at the Department of Clinical Genetics of the LUMC.

Dasatinib treatment

Several Dasatinib (Toronto Research Chemicals Cat# D193600) concentrations were used to investigate treatment options in vitro. Dasatinib (stock solution concentration of 20 mM in DMSO) was dissolved in Amniochrome® Pro Medium (Lonza Group Ltd, Basel, Switzerland) to obtain concentrations ranging from 2 nM to 2 μM and used in experiments as described. As control, we used standard Amniochrome® Pro Medium.

Cell proliferation

The effect of Dasatinib on UM cell viability was measured by mitochondrial function using the water-soluble tetrazolium salt (WST-1) assay (Roche Diagnostics, Indianapolis, IN, USA). In short, 96-well plates were filled with 1200 uveal melanoma cells per well, with either regular medium (control) or Dasatinib solutions (200nM to 2μM), and placed in an incubation chamber (20% O_2, 5% CO_2, 37°C). After the indicated incubation time (1, 2, or 3 days) 10ul of WST-1 reagent was added to each well and absorbance was measured at 450nm (n=8) on a multiwell spectrophotometer (Perkin Elmer, Wellesley, MA).

PamGene tyrosine-kinase array

To ascertain tyrosine-kinase activity in lysates of UM tissue and primary cultures, the PamChip-4 of PamGene B.V. ('s Hertogenbosch, The Netherlands) was used, as described previously [13]. In short, this array consists of 144 peptides spotted on a porous carrier. The 144 peptides are known tyrosine-kinase substrates and phosphorylation of these peptides by cell lysates can be monitored because of a generic antibody that recognizes phosphorylated tyrosine. Each chip consists of four arrays, so four lysates can be measured at the same time. In this experiment, we incubated a control lysate with and without addition of Dasatinib at an end concentration of 200nM on an array following the manufacturer's protocol.

Western blot analysis

Cell lysates were obtained by lysing cells of UM cryosamples and primary UM cultures (with and without Dasatinib treatment) in M-PER Mammalian Protein Extract Reagent (Pierce, Rockford, IL, USA), supplemented with 1% Halt Protease Inhibitor Cocktail, EDTA-free (Pierce) and 1% Halt Phosphatase Inhibitor Cocktail (Pierce). Protein concentrations were measured by using the BCA Protein Assay kit (Pierce).

Cell lysates, containing the same amount of protein, were mixed with equal volumes of 6× sample loading buffer, boiled for 10 min, cooled on ice, and then analyzed by 10% SDS polyacrylamide-gel electrophoresis (SDS-PAGE). Separated proteins were transferred to a PVDF membrane (Millipore). Subsequently, the membrane was blocked with Li-Cor blocking buffer for 1 h at room temperature and incubated with primary antibodies (rabbit anti-Src, rabbit anti-Erk1/2, mouse anti-pErk1/2, rabbit anti-GAPDH (loading control) (Cell Signalling, Boston, USA) followed by an infrared labeled secondary antibody (goat anti-rabbit800 and goat anti-mouse680 (LI-COR, Lincoln, USA). Labeled PVDF membranes were scanned with the Li-Cor scanner and quantification of proteins was performed using the Li-Cor software.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 software (SPSS Inc. Chicago, Illinois, USA). One-way ANOVA was used to analyze significant difference in cell proliferation of treated and untreated cells. The significance of differences in Src and ERK1/2 expression between samples with different histologic and molecular parameters was determined using unpaired T-tests. Additionally, all parameters were analyzed for survival and prediction of metastatic disease using the Kaplan-Meier analysis, log rank testing, cox regression and multivariate analysis. P-values for categorical parameters (associations) were obtained by Independent-Samples T Test (t-test for Equality of Means) and for the numerical data by Pearson correlation (2-tailed). $P < 0.05$ was considered statistically significant.

RESULTS

Inhibition of UM growth by Dasatinib

The application of the experimental Src inhibitors PP1 and PP2 has already shown that Src inhibition substantially reduces cell proliferation in UM cell lines [13]. The aim of the study was to analyze the efficacy of the commercially-available Src kinase-inhibitor Dasatinib in primary UM cell cultures. UM cell viability was significantly reduced by Dasatinib in a dose-dependent manner in five out of eight UM cell cultures (Fig. 1). Three cultures appeared Dasatinib resistant and displayed no growth arrest.

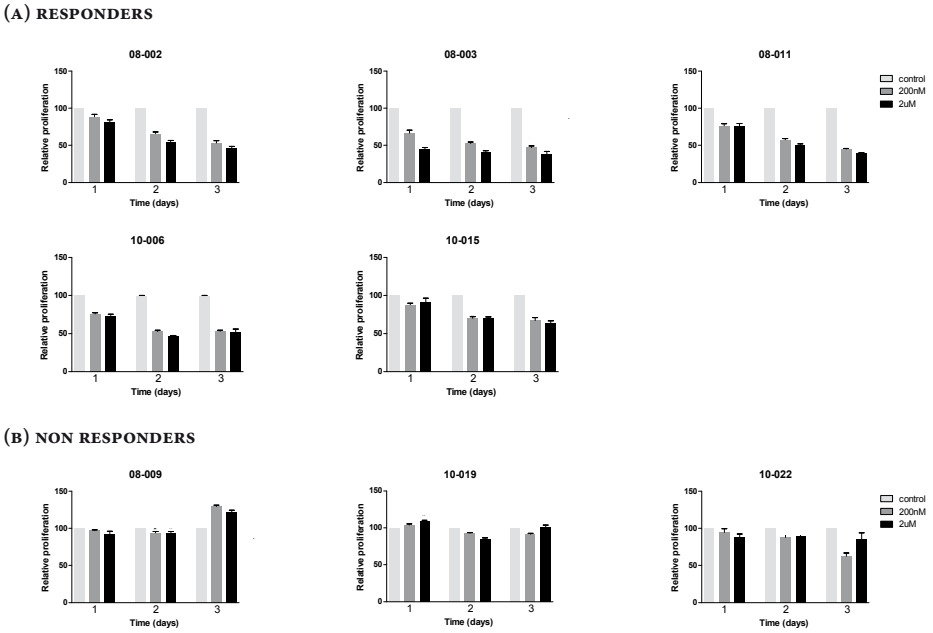

FIGURE 1. Cell viability of primary UM cell cultures treated with Dasatinib.
Cell viability was measured at 3 consecutive days. Each time point embodied a control culture and two Dasatinib treatments (200 nM and 2 μM). The untreated control culture is set at 100% and in five out of eight cultures viability was reduced upon Dasatinib treatment.

MAPK involvement

In order to investigate the molecular mechanisms by which Dasatinib induces growth arrest, we compared MAPK activation (pERK) and Src kinase activity in responders and non-responders. Primary cell cultures were exposed to two concentrations of Dasatinib. Basal expression of both Src and ERK varied widely in UM tissue cultures but revealed no correlation with treatment efficacy. Neither Src nor ERK expression was reduced after Dasatinib treatment (Fig. 2a). However, Dasatinib treatment significantly reduced activated ERK (pERK) in UM cell cultures (Fig. 2a and b). The degree of pERK down-regulation varied widely and tended to be highest in the responders as compared to the non-responders. Furthermore, the degree of ERK activation, prior to treatment, also seemed to be higher in the responding cultures (not shown).

Src and pERK expression in primary UM samples

In order to validate the correlation between Src and MAPK activation, Src and pERK expression in 36 UM was analyzed (Table 1). Expression levels of Src protein and ERK/pERK were quantified with Western analysis. All samples expressed Src (range of 1.17-55.83 integrated

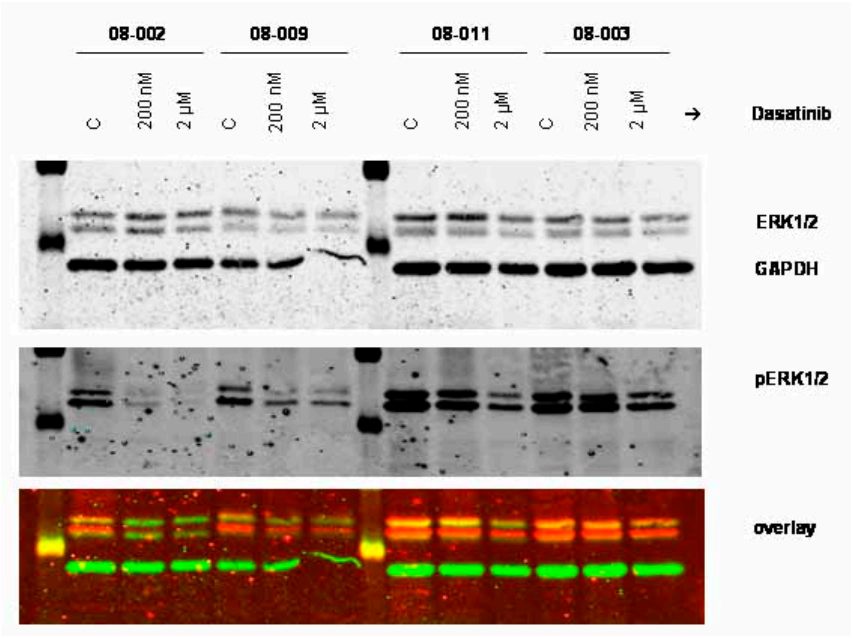

FIGURE 2A. ERK/pERK analysis in UM cell cultures treated with Dasatinib.
ERK/pERK analysis of 4 representative UM cell cultures treated for 48 hours with two concentrations of Dasatinib. Simultaneous hybridization with total ERK (top) and pERK (middle) specific antibodies allows quantification of pERK relative to ERK presence (bottom). Red in the overlay represents high pERK while yellow represents reduced pERK and absence of pERK is represented by a green signal.

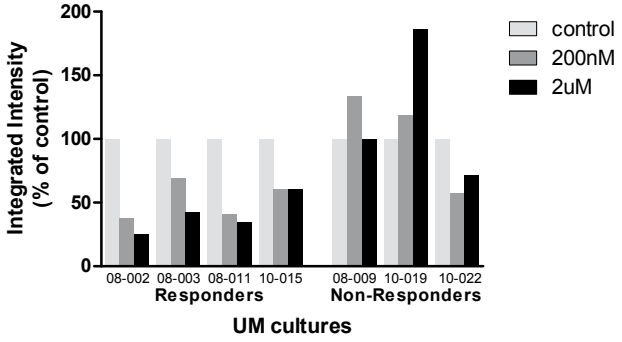

FIGURE 2B. ERK/pERK quantification in UM cell cultures treated with Dasatinib.
Activated ERK (pERK) in UM cell cultures after Dasatinib treatment in the responders and the non-responders. Relative ERK activation (pERK/ERK) is normalized to untreated control cultures.

TABLE 1. Distribution of Src expression and clinical and pathological features of 36 cases of uveal melanoma. Src expression was determined using Western blot analysis.

	Total		Associations		
Clinicopathologic factors	**N**	**%**	**Src expression**	**Standard Deviation**	**P- value**
Gender					.10
Male	18		19,64	10,20	
Female	18		30,97	14,46	
Eye					.98
Right	24		25,26	13,59	
Left	12		25,39	14,24	
Histopathologic cell type					.33
Spindle	8		21,14	7,99	
Mixed/ Epithelioid	28		26,49	14,72	
CB involvement					**.03**
Not present	21		21,17	10,74	
Present	15		31,10	15,38	
Scleral invasion					
None	8		25,92	15,85	
Intrascleral	24		25,73	12,74	
Extrascleral	4		21,49	17,55	
Chromosome 3 status					**.001**
Disomy 3	11		14,21	9,64	
Monosomy 3	25		30,18	12,25	
Metastasis					.10
No	23		22,49	12,31	
Yes	13		30,28	14,84	
Death					.20
Yes, due to other cause	3		29,37	17,72	
Yes, due to metastasis	11		29,32	16,02	
No	22		22,74	11,77	
GNA11					.99
No	17		25,32	17,00	
Yes	19		25,29	10,16	
GNAQ					.89
No	17		24,48	10,46	
Yes	16		23,82	16,34	
GNA11/GNAQ status					.75
GNA11	19		25,29	10,16	
GNAQ	16		23,82	16,34	
			Correlations		
			Coefficient		P-value

Age at diagnosis (year)	36	0.41	.01
Tumor diameter (mm)	36	0.19	.26
Tumor thickness (mm)	36	-0.04	.82
Erk expression (SD)	**36**	**0.40**	**0.02**
pErk expression (SD)	**36**	**0.71**	**<.001**

Data indicate the mean ± SD for the categorial parameters and correlation coefficients for the numerical variables.

P-values for categorical parameters (associations) were obtained by Independent-Samples T Test (t-test for Equality of Means) and for the numerical data by Spearman correlation (2-tailed).

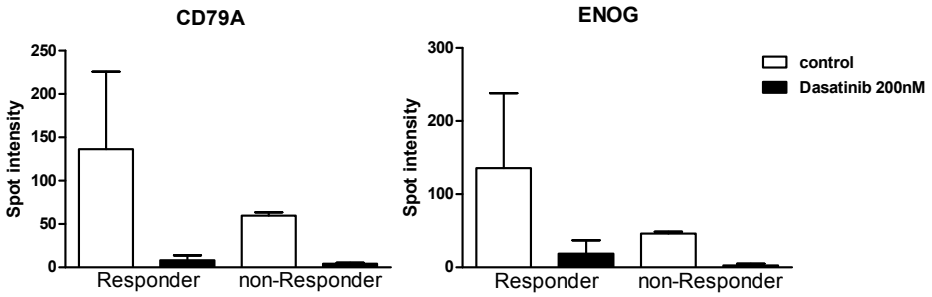

FIGURE 3A. Src kinase activity in UM cultures.

Phosphorylation of ENOG and CD79A peptides on the kinase array represent Src kinase activity in UM cell lysates and this activity is lost upon incubation with Dasatinib. Kinase activity of treated and untreated lysates are plotted for Dasatinib responders (n=3) and non-responders (n=3).

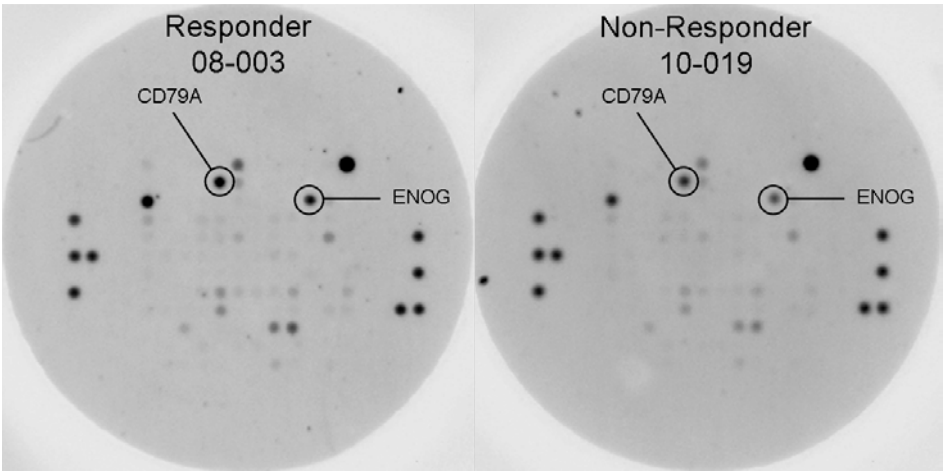

FIGURE 3B. Src kinase activity of Dasatinib responder and non-responder.

Representative kinase arrays of UM that were used for in vitro analysis. The cells of the tumor on the left responded with growth arrest whereas the cell culture of the tumor on the right did not respond to Dasatinib treatment.

intensity), ERK (range of 0.1-22.0 integrated intensity) and pERK (range of 0.84-13.29 integrated intensity) at variable levels (Fig 4a). Src expression was not correlated with ERK expression but the level of pERK was highly correlated with the level of Src ($p<.001$) and Src level predicts pERK to large degree (correlation coefficient = 0.71) (Table 1). Though ERK expression was also found to be correlated with pERK ($p= 0.017$) only part of the activation is predicted by ERK expression (correlation coefficient = 0.40).

Tyrosine-kinase activity

With kinase activity arrays Src activity in response to Dasatinib treatment was analyzed. Lysates of Dasatinib-treated UM cells all displayed reduced Src kinase activity, still a correlation with treatment efficacy was observed. UM cultures that responded to Dasatinib treatment with growth arrest presented a higher basal Src activity compared to the non-responding tumor cultures and hence a stronger reduction of kinase activity was observed (Fig 3a). Peptides on the kinase array, such as ENOG and CD79A, that are known substrates for Src kinase were highly phosphorylated by lysates of the Dasatinib sensitive UM cultures (Fig 3b).

Molecular mechanisms

Known molecular mechanisms in UM were analysed and compared to treatment efficacy. GNAQ and GNA11 mutations of Q209 represent early events in UM development that could be related to Src activity in UM. However, mutations in GNAQ/GNA11 are very common and hence only 2 wiltype tumor cultures were analyzed. The GNAQ/GNA11 wildtype UM were found to be present in both the responder and non-responder group. In addition, the GNAQ and GNA11 status of primary UM was assessed (n=36) and no correlation was detected between GNAQ/GNA11 mutation status and Src/pERK expression. Out of the 36 analyzed tumors, 19 displayed the GNA11 mutation and 16 contained the GNAQ mutation while for one sample, the sequence analysis failed.

Monosomy 3 is a late event and strongly correlated with metastasis development. Eight primary UM cultures were analyzed for chromosome 3 and all three non-responders displayed a normal chromosome 3 karyotype whereas three out of five responders displayed monosomy 3. These data suggest that Dasatinib efficacy and ERK activation through Src signaling may be correlated with tumor progression, and this was further analyzed using primary UM tissue.

Using 35 UM samples, we observed that the level of Src expression was significantly correlated with monosomy 3 ($p = 0.0014$): the mean Src expression in UM samples with monosomy 3 was much higher (mean of 29.87; SEM 2.452) than in UM samples with disomy 3 (mean of 14.91; SEM 3.249). Analysis for pERK demonstrated a similar trend, as expression of pERK was elevated in the UM samples with monosomy 3 (mean of 7.13; SEM 0.60) in comparison to UM samples containing 2 copies of chromosome 3 (mean of 5.05; SEM 0.92). This

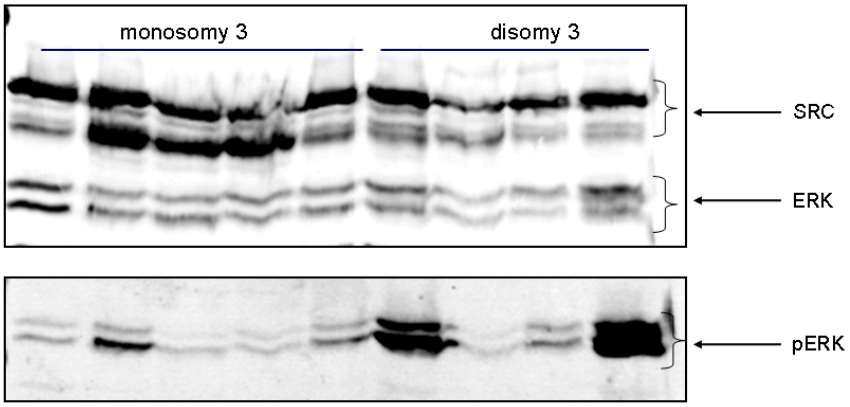

FIGURE 4A. Expression of Src, ERK and pERK in primary UM samples.
Representative example of the Western analysis that was conducted on 36 UM samples in order to analyze ERK activation and Src expression.

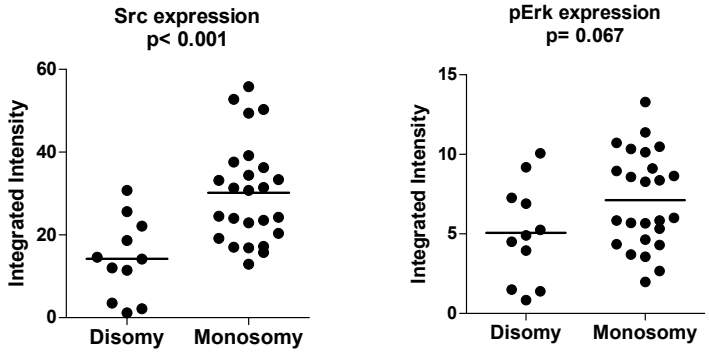

FIGURE 4B. Src/pERK expression and chromosome 3 status in UM tissue.
Protein expression levels of Src and pERK were measured and correlated with chromosome 3 status of 35 UM using unpaired T-test.

difference did however not reach statistical significance (p =0.068) (Fig. 4B). The analysis of the total amount of ERK1/2 expression and monosomy 3 revealed neither an association nor a trend (mean expression disomy 5.6, monosomy 4.6, p=0.750).

According to the TNM classification (7th edition) five tumors were stage I, four were stage IIA, ten were stage IIB, 14 were stage IIIA, one was stage IIIB, and one was stage IV. Besides ciliary body involvement (p=0.03), neither TNM classification, nor other clinical and histopathological parameters demonstrated an association with Src or pERK expression (Table 1).

Prognosis

Patient data and survival were updated till September 2010 and the mean follow-up at the time of analysis was 43 months (range, 3-121). During this period, 14 patients died, 11 due to metastatic disease. Two patients with metastasis were still alive at the end of follow-up and no patients were lost to follow up. According to Kaplan-Meier analysis and log rank testing, ciliary body involvement (hazard ratio 3.31, $p<0.05$), TNM classification (stage IV; hazard ratio 28.2, $p<0.05$) and monosomy 3 (hazard ratio 7.17, $p<0.05$), were significantly associated with a decreased survival. Src and pERK expression was not correlated to prognosis.

DISCUSSION

Src has previously been identified in UM cell lines as an important kinase involved in UM growth via the activation of MAPK signaling. Based on this, preclinical analysis with Dasatinib was performed, a Src family-kinase inhibitor that is used in the treatment of leukemia.

Using 8 primary UM cell cultures, we tested whether UM cell growth could be inhibited using Dasatinib. In about 60% of the UM cultures, cell proliferation was reduced after treatment with Dasatinib whereas in the remaining cultures, proliferation appeared unaffected. To investigate the underlying mechanism, MAPK activation in response to Dasatinib treatment was analyzed. In cell cultures that responded to Dasatinib with growth arrest, ERK activation (pERK) appeared to be higher prior to treatment and was also more strongly reduced upon Dasatinib treatment, compared to the non-sensitive cell cultures. To investigate the molecular mechanism of Dasatinib induced growth inhibition, the response was compared with the molecular characteristics of the UM. Tumors with GNAQ and GNA11 wildtype genotypes were detected in both the responders and the non-responders and could not distinguish the treatment groups. Monosomy 3 was however only detected in the responders while the non-responders all contained two copies of chromosome 3. This may indicate that a modifier of Src signaling is located on chromosome 3.

We also determined Src and MAPK activation status in 36 UM samples obtained after enucleation. A very strong correlation was detected between Src expression and ERK activation (pERK), supporting the notion that Src is associated with MAPK-driven UM proliferation and survival. Furthermore, we evaluated the possibility that Src upregulation and MAPK activation is due to somatic mutations in the heterotrimeric G protein alpha-subunit (GNAQ and GNA11) gene [10-13]. GNAQ/GNA11 mutations are the first oncogenic mutations in UM and were shown to occur in about 78% of tested primary UM samples [9,10]. In our UM cohort we detected an even higher abundance of GNAQ and GNA11 mutations (>90%) and hence we could not detect an association with Src mutation status and MAPK activation because a wild type group was lacking. Mutation of these genes in UM development appears to be an

early event and underlines the importance of secondary lesions as possible determinants of metastatic disease and elevated Src/MAPK activation.

Subsequent comparison of chromosome 3 status with Src and MAPK activation status in the primary tumors, and, in support of our in vitro analysis, revealed a significant association between Src expression and monosomy 3 (p= 0.0014). Src protein expression was approximately two-fold higher in monosomy 3 UM samples compared to disomy 3 UM samples. Monosomy 3 has been established as one of the most important molecular markers in UM patient survival and actually represents a late event in UM development [6,17,18]. This association supports in vitro data that suggest the presence of a Src modifier on chromosome 3. Recently, somatic mutations were identified in the gene encoding BRCA1-associated protein 1 (BAP1) on chromosome 3p21.1 which was significantly associated with metastatic occurrence [8]. BAP1 has been shown to possess tumor suppressor activity and mutations have been reported in some forms of cancer [19,20]. BAP1 mutation is a late event which may determine malignant conversion and should be analyzed for its role in Src/MAPK activation.

Previously, we reported a decreased Src activation in metastatic cell lines compared to primary UM cell lines [13]. However, in the same study residual Src activity was shown in UM metastasis tissue. Further analysis regarding the discrepancy between metastatic tissue and metastatic UM cell lines with regard to Src activity should be performed as loss of Src activity could be a potential cause of Dasatinib treatment resistance.

Uveal melanoma metastasizes almost exclusively to the liver and most current treatment options focus on this site [3]. Currently, conventional metastasis treatment including (systemic or intra-hepatic) chemotherapy or partial hepatectomy, provides no significant prolongation of survival compared to no treatment [4]. We propose the use of chromosome 3 status, ERK activation and Src kinase expression level of the primary UM and related metastases as a tool to select patients eligible for treatment with Dasatinib. In the future, prophylactic treatment of high risk patients could be an additional possibility hence biomarker analysis should be performed on both patients treated locally and patients treated with enucleation. Assessment of monosomy 3 by fine-needle-aspiration biopsy has been shown to be feasible in patients undergoing brachytherapy [21]. However, biopsies would only be clinically relevant if successful treatment options for metastases patients would become available.

In summary, we have shown that in selected UM, Dasatinib inhibits cell growth in vitro. The UM cultures that respond to Dasatinib treatment, differ in MAPK and Src kinase activity compared to non-responding primary UM cultures. Moreover, with monosomy 3 we may have revealed the underlying mechanism and hence we propose Dasatinib as a possible therapy for UM and we suggest monosomy 3, Src expression and MAPK activition as potential biomarkers for early adjuvant treatment.

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