The International Journal of Biochemistry & Cell Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Metastatic potential is determined early in synovial sarcoma development and reflected by tumor molecular features☆

Joanna Przybyl[a,b,g], Raf Sciot[c], Agnieszka Wozniak[d], Patrick Schöffski[d], Vanessa Vanspauwen[b], Ignace Samson[e], Janusz A. Siedlecki[a], Piotr Rutkowski[f], Maria Debiec-Rychter[b,*]

[a] Department of Molecular and Translational Oncology, Maria Sklodowska—Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland
[b] Department of Human Genetics, KU Leuven and University Hospitals Leuven, Leuven, Belgium
[c] Department of Pathology, KU Leuven and University Hospitals Leuven, Leuven, Belgium
[d] Laboratory of Experimental Oncology, Department of Oncology, KU Leuven, and Department of General Medical Oncology, University Hospitals Leuven, Leuven, Belgium
[e] Department of Orthopedic Surgery, University Hospitals Leuven, Leuven, Belgium
[f] Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Sklodowska—Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland
[g] Postgraduate School of Molecular Medicine, Warsaw Medical University, Warsaw, Poland

ARTICLE INFO

Article history:
Received 15 January 2014
Received in revised form 8 May 2014
Accepted 9 May 2014
Available online xxx

Keywords:
Synovial sarcoma
Metastasis
Gene expression profiling
Genomic complexity
Aurora kinase A
Kinesin family member 18A

ABSTRACT

Introduction: Synovial sarcoma (SynSa) is an aggressive mesenchymal tumor, comprising approximately 10% of all soft tissue sarcomas. Over half of SynSa patients develop metastasis or local recurrence, but the underlying molecular mechanisms of the aggressive clinical behavior remain poorly characterized.
Materials and methods: Sixty-four frozen tumor specimens from 54 SynSa patients were subjected to array comparative genomic hybridization (aCGH) and gene expression profiling. The examined set of tumor specimens included 16 primary tumors from untreated patients who did not develop metastasis/local recurrence (SynSa1 group), 26 primary tumors from untreated patients who developed metastases or local recurrence during follow-up (SynSa2 group), and 22 metachronous metastatic/recurrent SynSa tumors (SynSa3 group).
Results: AURKA and *KIF18A*, which play important roles in various mitotic events, were the two most up-regulated genes in SynSa2 and SynSa3 groups compared to the SynSa1 group. Expression profiles of SynSa2 and SynSa3 tumors did not show any significant differences. Analysis of genomic index (GI) based on aCGH profiles demonstrated that the SynSa1 group consisted of tumors with significantly less complex genomes compared to SynSa2 and SynSa3 groups. There was no significant difference in genome complexity between SynSa2 and SynSa3 tumors.
Conclusions: Primary SynSa tumors from patients who develop metastases or local recurrence share common molecular features with metastatic/recurrent tumors. Presented data suggest that the aggressive clinical SynSa behavior is determined early in tumorigenesis and might be related to impaired regulation of mitotic mechanisms.
This article is part of a Directed Issue entitled: Rare Cancers.

1. Introduction

Synovial sarcoma (SynSa) is an aggressive type of mesenchymal tumor, comprising approximately 10% of all soft tissue sarcomas. The age-standardized incidence rates per million population range from 0.5 to 1.3 (Wibmer et al., 2010; Ducimetière et al., 2011; Cancer Research UK, 2013). SynSa occurs in patients at any age, but mainly in adolescents and young adults, with vaguely higher prevalence in males (Suurmeijer et al., 2013). Approximately 80% of primary SynSa arise in the extremities, most commonly in

Abbreviations: Ct, threshold-cycle; lincRNA, long intergenic non-protein coding RNA; GEO, gene expression omnibus.

☆ This article is part of a Directed Issue entitled: Rare Cancers.

* Corresponding author. Tel.:+32 16 347218; fax: +32 16 346080.
E-mail address: maria.debiec-rychter@med.kuleuven.be (M. Debiec-Rychter).

http://dx.doi.org/10.1016/j.biocel.2014.05.006
1357-2725/

the para-articular regions (Eilber and Dry, 2008). However, they virtually never develop within the joint and have no biological or pathological relation with normal synovium (Eilber and Dry, 2008). SynSa is classified as a sarcoma of unknown origin, but recent findings have pointed to either a neural (Ishibe et al., 2008), myogenic (Haldar et al., 2007), or multipotent mesenchymal stem cell origin (Naka et al., 2010; Garcia et al., 2011).

The molecular mechanism of SynSa development is only partially known. Over 90% of SynSa are characterized by the t(X;18)(p11.2;q11.2) translocation, which results in the formation of *SS18-SSX1* fusion in 2/3 of cases or *SS18-SSX2* fusion in the majority of remaining cases. SS18-SSX1/2 chimeric proteins particularly affect cell growth, cell proliferation, TP53 pathway, and chromatin remodeling mechanisms, contributing to SynSa oncogenesis (reviewed in Przybyl et al., 2012a).

Over half of SynSa patients develop local recurrences or distant metastases, most commonly in the lung. SynSa is characterized by slow tumor growth and high incidence of late metastases, which frequently develop after more than 5 years from the initial diagnosis (Gofman et al., 2007; Krieg et al., 2011).

Recently published results of genomic and gene expression profiling in SynSa show that the metastatic outcome may be predicted based on the molecular features of the primary tumors (Lagarde et al., 2013). In the present study, we have performed array comparative genomic hybridization (aCGH) and gene expression analysis of metastatic and recurrent SynSa tumors to evaluate if the aggressive course of disease is correlated with the accumulation of specific molecular cytogenetic and/or gene expression anomalies.

2. Materials and methods

2.1. Patients

Patients were diagnosed and treated at the University Hospitals Leuven, Belgium ($n = 52$) and the Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland ($n = 2$). The non-interventional, retrospective study was approved by the Committees for Biomedical Ethics of both institutes. Sixty four frozen tumor specimens from 54 SynSa patients [34 males; median age: 38 years (range: 5–85 years)] were included in this study (Table 1). The median follow-up for all patients was 54 months (range: 4–281 months). The examined set of specimens included 16 primary tumors from untreated patients who did not develop metastasis/local recurrence ("SynSa1 group"; median follow-up 119 months), 26 primary tumors from untreated patients who developed metastases or local recurrence during follow-up ("SynSa2 group"; median follow-up 36 months), and 22 metachronous metastatic/recurrent SynSa tumors ("SynSa3 group"; median follow-up 46 months). For eight patients from SynSa2 group, the primary tumor specimens were paired with available metastatic/recurrent tumor specimens.

All tumors were diagnosed as typical SynSa by means of morphology and routine immunohistochemistry. Tumors with >80% of round cell component were classified as poorly differentiated. Fusion transcript detection by reverse transcription polymerase chain reaction was performed in all tumor specimens as described before (Przybyl et al., 2012b).

2.2. Gene expression profiling

Total RNA was extracted from frozen specimens using the miRNeasy Mini Kit (Qiagen) with DNase treatment using RNase-free DNase Set (Qiagen). RNA quality was checked using Bio-Rad Experion system (Bio-Rad Laboratories). RNA of sufficient integrity and purity was obtained from 57 of tumor specimens. Gene expression analysis was performed using Human Gene Expression 4×44K v2 microarrays (G4845A) according to the one-color microarray-based expression analysis protocol (Agilent Technologies). Gene expression data were analyzed using Gene Spring GX software with default workflow parameters for Agilent one-color expression microarrays (Agilent Technologies). One-way ANOVA test was performed and p-values were adjusted using the Benjamini–Hochberg procedure.

2.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

Five hundred nanogram of total RNA were reverse transcribed with oligo$(dT)_{12-18}$ primers and random hexamers using High-Capacity cDNA Reverse Transcription kit (Life Technologies). The qRT-PCR was carried out in duplicate to detect *AURKA* and *KIF18A* expression levels, using TaqMan Gene Expression Assays Hs01582072_m1 and Hs01015428_m1, respectively (Life Technologies), according to manufacturer's protocol. Threshold-cycle (Ct) values were generated for each sample in the SDS 2.1 software (Life Technologies). The qRT-PCR data were analyzed using DataAssist v3.01 software (Life Technologies), which calculates the relative amount of RNA for each gene using the comparative Ct ($\Delta\Delta$Ct) method (Schmittgen and Livak, 2008). Mean expression level of two reference genes *POP4* and *EIF2B1* served as endogenous control. Negative controls were also included in every step of the procedure, and cDNA from HS-SY II SynSa cell line (RIKEN BioResource Center Cell Bank) served as an inter-plate calibrator. Fold change data were calculated as $2^{-\Delta\Delta Ct}$.

2.4. Array comparative genomic hybridization (aCGH)

Genomic DNA was extracted from the frozen tumor specimens using High Pure PCR Template Preparation Kit (Roche). DNA of sufficient quality was obtained from 61 tumor specimens. One microgram of genomic DNA was hybridized to SurePrint G3 Human CGH 4×180K microarrays (G4449A) according to the manufacturer's protocol (Agilent Technologies). The aCGH data were analyzed using Agilent Genomic Workbench 7.0.4.0. software (Agilent Technologies) and the ADM-2 algorithm was applied to identify DNA copy number aberrations. Copy number gain, loss and amplification were defined as described before (Lagarde et al., 2013). Based on aCGH results, the genomic index (GI) was calculated for each tumor specimen as follows: $GI = A^2/C$, where A is the total number of alterations (segmental gains and losses of minimum 500 probes in the region), and C is the number of involved chromosomes (Lagarde et al., 2012).

2.5. Western blot analysis

To validate the expression of AURKA on protein level, total proteins were extracted from 16 frozen tumor specimens and evaluated by Western blot analysis, as previously described (Floris et al., 2013). The antibodies used were monoclonal anti-aurka A and anti-β-actin (both from Sigma-Aldrich).

2.6. Statistical analysis

Progression-free survival (PFS) was calculated from the date of initial diagnosis to the date of first metastasis or local recurrence, or the last follow-up for patients without progressive disease. Multivariate analysis was performed using Cox proportional hazards model (Statistica 10, Statsoft). The one-way analysis of variance (ANOVA) was used to analyze the significance of differences in age, tumor size and mitotic index between SynSa groups. Contingency tables were analysed using Chi-square test.

Please cite this article in press as: Przybyl J, et al. Metastatic potential is determined early in synovial sarcoma development and reflected by tumor molecular features. Int J Biochem Cell Biol (2014), http://dx.doi.org/10.1016/j.biocel.2014.05.006