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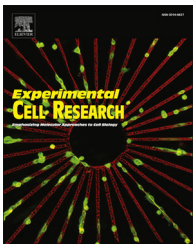

Research Article

Growth rate of late passage sarcoma cells is independent of epigenetic events but dependent on the amount of chromosomal aberrations

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ABSTRACT

Soft tissue sarcomas (STS) are characterized by co-participation of several epigenetic and genetic events during tumorigenesis. Having bypassed cellular senescence barriers during oncogenic transformation, the factors further affecting growth rate of STS cells remain poorly understood. Therefore, we investigated the role of gene silencing (DNA promoter methylation of *LINE*-1, *PTEN*), genetic aberrations (karyotype, *KRAS* and *BRAF* mutations) as well as their contribution to the proliferation rate and migratory potential that underlies "initial" and "final" passage sarcoma cells. Three different cell lines were used, SW982 (synovial sarcoma), U2197 (malignant fibrous histiocytoma (MFH)) and HT1080 (fibrosarcoma). Increased proliferative potential of final passage STS cells was not associated with significant differences in methylation (*LINE*-1, *PTEN*) and mutation status (*KRAS*, *BRAF*), but it was dependent on the amount of chromosomal aberrations. Collectively, our data demonstrate that these fairly differentiated/advanced cancer cell lines have still the potential to gain an additional spontaneous growth benefit without external influences and that maintenance of increased proliferative potential towards longevity of STS cells (having crossed senescence barriers) may be independent of overt epigenetic alterations.

Introduction

Soft tissue sarcomas (STS) are a heterogeneous group of rare tumors with mesenchymal origin. Several epigenetic and genetic alterations have been reported to co-participate in STS tumorigenesis [1–4]. Bypassing cellular senescence barriers has been implicated as a critical event regulating tumorigenesis in several cancers including sarcomas [5,6]. Factors further affecting survival of STS cells subsequent to the bypassing of cellular senescence barriers have remained poorly studied.

Earlier studies have hypothesized a role of the DNA hypermethylation status in the pathogenesis of soft tissue sarcomas.

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Differential promoter methylation of *RASSF1A* (26%), *DAP kinase* (17%), *MGMT* (15%), *GSTP*1 (14%), *PTEN* (12%), *p*16 (9%), *hMLH*1 (9%), *hMSH*2 (8%), *p*14 (3%) and *RB* (2%) has been reported [1]. Interestingly, for *PTEN* in STS, promoter methylation (13%) was more frequent than *PTEN* point mutations (4%) and *PTEN* homozygous deletion (2%) [3]. About 17% of the human genome consists of long interspersed nuclear elements (LINE-1) [7]. The methylation status of LINE-1 serves as an indicator of overall global methylation level [8,9]. Due to the fact that LINE-1 hypomethylation could result in chromosomal instability and is associated with shorter survival among colon cancer patients and serves as a biomarker for bladder cancer risk [10,11], it could also play a particular role in the pathogenesis of some STS and could show an alteration after a long period of cell cultivation

The frequencies of *RAS* mutations in STS varied from 0% to 44% depending on the ethnic and genetic heterogeneity of the study population [12], whereas the frequency of *BRAF* mutations was only 5% [13]. Furthermore, chromosomal aberrations have been demonstrated to be prognostically important in STS. Mertens et al. identified five independent cytogenetic predictors of adverse outcome: breakpoints in the chromosome regions 1p1, 1q4, 14q1 and 17q2 as well as duplication of regions in 6p1-p2 [14]. An increasing metastatic risk of STS was reported to be associated with increasing involvement of specific cytogenetic variables, even when different histopathological types were studied separately. Collectively, these observations suggest that contribution of DNA methylation and oncogenic mutations (*KRAS*, *BRAF*) may be negligible in comparison to chromosomal aberrations during oncogenic transformation of STS. Yet, the relevance of these factors in affecting growth rate of STS cells subsequent to oncogenic transformation remains unclear.

In this study we used three different STS cell lines to investigate both the role of epigenetic (*LINE*-1, *PTEN* methylation status), and the genetic parameters (karyotype, *KRAS*, *BRAF* mutations). Furthermore we analyzed their possible contribution to the survival (proliferation and migration) that underlies initial and final passage sarcoma cells.

Materials and methods

Cell culture

The human cell lines SW982 synovial sarcoma and HT-1080 fibrosarcoma cell line (Cell Line Service—CLS, Eppelheim, Germany), were grown in DMEM supplemented with 10% FCS (Thermo Fisher Scientific Inc., Waltham, MA, USA) and 1% penicillin/streptomycin (PAA laboratories, Pasching, Austria). U2197 malignant fibrous histiocytoma cell line (Cell Line Service —CLS, Eppelheim, Germany) was grown in MEM supplemented with 20% FCS (Thermo Fisher Scientific Inc., Waltham, MA, USA), 1% penicillin/streptomycin and 0.135% Sodium Bicarbonate (PAA laboratories, Pasching, Austria). Cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2.

Cell size and morphology

Cells were incubated in 1% trypsin (PAA laboratories, Pasching, Austria) for detachment and cell size was measured at least on 10 different days with a CASY®-1 TT cell counter (Schärfe System, Reutlingen, Germany) according to the supplier's guide. Morphologic evaluation were made via a Zeiss Axioskop 2 Plus microscope (Carl Zeiss Jena GmbH, Jena, Germany) and pictures were taken using the software Zeiss Axio Vision 3.1. (Carl Zeiss Jena GmbH, Jena, Germany).

Cell viability MTT-assay

The cell viability was measured via MTT assay according to the standard protocol as described elsewhere [15]. Short: cells were seeded in a 96well microtiterplate (clear flat bottom, Corning, Omnilab, Bremen, Germany) at a density of 2×10^3 cells per well in 150 µl DMEM and incubated at 37 °C and 5% CO_2 for 0, 24, 48, 72 and 96 h. 50 µl of 0.5 mg/ml MTT (Sigma, St. Louis, USA) was added for 3 h. MTT (3-(4,5 Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a yellow dye that is reduced to purple formazan in the mitochondria of living cells. After addition of 200 µl DMSO (Carl Roth GmbH, Karlsruhe, Germany) and 25 µl glycine buffer (containing 0.1 M glycine, 0.1 M NaCl, pH 10.5, adjusted with NaOH) per well, cells were lysed. The absorbance of this solution was measured with an ELISA Plate Reader (BioTek Instruments, Bad Friedrichshall, Germany) at 562 nm, using the software KC4. A growth curve was generated and the doubling rate was calculated by the algorithm provided by http://www.doubling-time.com.

Scratch assay

Cells were seeded at a density of 1.5×10^4 cells per well in a ibidi culture-insert (µ-Dish35 mm, low Culture-Insert, ibidi, Martinsried, Germany,) in 80 µl DMEM and incubated at 37 °C and 5% CO_2 for 24 h. Inserts were removed and pictures were taken immediately and depending on the speed of migration; after 48 (HT-1080) and 96 (SW982, U2197) hours. Number of pixels of the cell-free area was measured using the software Photoshop (Adobe Systems, San Jose, California).

Karyotyping

Cell cultures were incubated with 1 µg colcemide (Gibco, Karlsruhe, Germany) for 80 min and were than harvested from flask applying trypsin-EDTA (0.05/0.02 w/v) (Biochrome, Berlin, Germany) for 2–4 min. After transferring to tube and centrifugation (170 g for 10 min) cells were incubated in 0.56% KCl hypotonic solution for 20 min and subsequently fixed and washed using a 3:1 methanol–glacial acetic acid fixative. After spreading on slides and air drying samples were stained in 0.025% quinacrine hydrocloride (Sigma, Steinheim, Germany) for 20 min. Q bands were visualized on a Zeiss Axioskop 2 fluorescent microscope and 100 metaphases per sample were analyzed in Ikaros software (Metasystems, Altlussheim, Germany).

Genomic DNA extraction

Genomic DNA was extracted from cells using QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations.