Expression of human Piwi-like genes is associated with prognosis for soft tissue sarcoma patients

Thomas Greither1, Franziska Koser2†, Matthias Kappler2, Matthias Bache3, Christine Lautenschläger4, Steffen Göbel5, Hans-Jürgen Holzhausen5, Sven Wach7,8, Peter Würl9 and Helge Taubert7,8,10*

Abstract

Background: Argonaute genes are essential for RNA interference, stem cell maintenance and differentiation. The Piwi-like genes, a subclass of the Argonaute genes, are expressed mainly in the germline. These genes may be re-expressed in tumors, and expression of the Piwi-like genes is associated with prognosis in several types of tumors.

Methods: We measured the expression of Piwi-like mRNAs (Piwi-like 2–4) in 125 soft tissue sarcoma (STS) samples by qPCRs. Statistical tests were applied to study the correlation of expression levels with tumor-specific survival for STS patients.

Results: In multivariate Cox’s regression analyses, we showed that low Piwi-like 2 and Piwi-like 4 mRNA expression were significantly associated with a worse prognosis (RR = 1.87; p = 0.032 and RR = 1.82; p = 0.039). Low expression of both genes was associated with a 2.58-fold increased risk of tumor-related death (p = 0.01). Piwi-like 4 and combined Piwi-like 2 and 4 mRNA levels correlated significantly with prognosis (RR = 3.53; p = 0.002 and RR = 5.23; p = 0.004) only for female but not for male patients. However, combined low Piwi-like 2 and 3 transcript levels were associated with worse survival (RR = 5.90; p = 0.02) for male patients.

Conclusions: In this study, we identified a significant association between the expression of Piwi-like 2 and 4 mRNAs and the tumor-specific survival of soft tissue sarcoma patients. Furthermore, a connection between sex and the impact of Piwi-like mRNA expressions on STS patients’ prognosis was shown for the first time.

Background

The Piwi (P-element-induced wimpy testis) family is a subclass of the Argonaute gene/protein family characterized by their homology and the occurrence of PAZ and Piwi domains [1,2]. PIWI proteins play important roles in stem cell self-renewal, spermatogenesis, transposon and RNA silencing, translational regulation, and chromatin remodeling in various organisms [3]. Cox and coworkers identified the first Piwi gene in Drosophila [4]. Piwi is required for the asymmetric division of germ-line stem cells (GSCs) to produce and to maintain a daughter GSC (stem cell self-renewal). In addition, homologues of the Piwi gene have been identified in Caenorhabditis elegans, Arabidopsis and humans, but not in bacteria or yeast, suggesting that Piwi has a stem cell-related function existing only in multicellular organisms. Piwi-like genes also play a role in adult somatic stem cells, such as human hematopoietic stem cells and mouse mesenchymal stem cells [5,6]. Four members of the human Piwi gene family, Piwi-like 1 (Hiwi, Piwil1), Piwi-like 2 (Hili, Piwil2), Piwi-like 3 (Piwil3) and Piwi-like 4 (Hiwi2, Piwil4), have been described so far [7]. The genes have been mapped to different chromosomal regions. Piwi-like 1 and Piwi-like 2 are located on chromosome 12 (12q23-12q24.33) and chromosome 8 (8p21). Piwi-like 3 and Piwi-like 4 have been mapped to chromosome 22 (22q11.2) and chromosome 11 (11q12). An increasing number of publications have demonstrated that members of the Piwi family may be re-expressed in malignant tumors. Expression of these genes may correlate with tumor behavior and patient prognosis. Elevated protein expression of PIWIL1 is...
associated with a poor prognosis in seminoma, gastric cancer, esophageal cancer and glioma [8-11]. PIWIL2 protein may be detected in various stages of cervical squamous cell carcinoma and breast adenocarcinoma, as well as in metaplastic epithelial cells and histologically normal-appearing tissues adjacent to breast and cervical tumors [12-14]. Additionally, high levels of PIWIL2, PIWIL3 and PIWIL4 proteins are correlated with an elevated risk of colon cancer [15]. Li and coworkers also showed that increased PIWIL4 protein levels are significantly associated with the risk of metastasis and prognosis in colon cancer patients [15].

Protein levels and mRNA levels are not necessarily correlated [16]. Reasons for this could be different mRNA stability, mRNA degradation, and posttranscriptional regulation mechanisms. There are regulatory layers of the transcriptome in which RNA-binding proteins (RBPs), noncoding regulatory RNAs (ncRNAs) and messenger RNAs (mRNAs) can interact [17].

However, there have been comparatively few studies evaluating the impact of Piwi-like (PIWI) gene expression in human cancers. In patients with gastric cancer and soft tissue sarcoma (STS) and in male pancreatic carcinoma patients, the levels of mRNA expression of Piwi-like 1 are correlated with prognosis [9,18,19]. Expression of the Piwi-like 2 gene has been detected in many different human tumors, including prostate, breast, gastrointestinal, ovarian and endometrial cancers [20]. However, no data have been published about Piwi-like 3 and Piwi-like 4 mRNA expression levels in human tumors. A search in the Oncomine database revealed an expression of Piwi-like 3 in carcinomas of the breast, colon, ovary and brain and for Piwi-like 4 in carcinomas of the breast, liver and brain. In this study, we investigated Piwi-like mRNA expression in patients with STS. We attempted to correlate Piwi-like mRNA levels with clinical factors such as tumor size and with tumor-specific survival. We also performed a sex-specific analysis of the prognostic impact of Piwi-like mRNA expression in patients with STS.

Methods

Patients

In this study, tumor tissue samples of 125 patients with STS were analyzed. All patients underwent surgical resection in the Department of Surgery, Martin-Luther-University Halle-Wittenberg and the Department of Surgery 1, University of Leipzig, Germany. The cohort has been described in previous studies [21]. All diagnoses were verified by an experienced pathologist (HJH) according to the UICC system. All patients gave written informed consent. The study was approved by the Ethic Committee of the Medical Faculty of the Martin-Luther-University Halle-Wittenberg and it was performed in compliance with the Helsinki Declaration. Median patient age at the time of surgery was 58 years (range 14–87 years). Median follow-up time was 32 months (range 2–201 months after primary tumor resection). Forty-nine patients experienced locoregional recurrence, 10 patients developed distant metastases and 61 patients died during the observation time. All tumor samples were collected before radio- or chemotherapy. For clinical and histopathologic parameters, please refer to Table 1.

RNA isolation

Ten to twenty tumor tissue slices (30 μm) were used for RNA isolation with Trizol reagent according to the manufacturer’s protocol (Invitrogen, Karlsruhe, Germany). Briefly, RNA was extracted with phenol/chloroform and precipitated by isopropanol. Any remaining DNA traces were removed by DNAse I digestion (Qiagen, Hilden, Germany). The remaining pellet was washed twice in ice-cold ethanol and resuspended in 30 μl of RNAse-free water. RNA concentration was assessed with a Nanodrop ND-1000 Spectrophotometer (Thermo Scientific, Karlsruhe, Germany). RNA was stored at −80 °C.

cDNA synthesis

Two micrograms of RNA were used for cDNA synthesis. cDNA was synthesized with the RevertAid H Minus First strand cDNA Synthesis Kit according to the manufacturer’s protocol (Fermentas, St. Leon-Rot, Germany).

qPCR

Piwi-like gene expression was measured using TaqMan Gene expression assays (Piwi-like 2: Hs01032719; Piwi-like 3: Hs00908837; Piwi-like 4: Hs00895218) according to the manufacturer’s protocol (Applied Biosystems, Darmstadt, Germany). The recognition sites of primers within the Piwi-like mRNAs are given in Additional file 1: Figure S1. Quantitative RT-PCR reactions were performed with the HotStart-Taq Polymerase Kit (Qiagen, Hilden, Germany) in a real-time cycler (LTF, Wiesbaden; Germany). HPRT (hypoxanthine phosphoribosyl transferase) expression was measured with the following primers (HPRT fw: 5′-TTGCTGACCTGCTGGATTAC-3′; rw: 5′-CTTGGCGACCTTGACCATCTT-3′) using Maxima SYBR-Green qPCR Master Mix (Fermentas, St. Leon-Rot, Germany) and served as reference gene. All measurements were carried out from the same cDNA aliquot within a short time period to ensure comparable conditions. Gene expression was normalized to HPRT mRNA expression. Quantification was performed using self-established plasmid standards for Piwi-like 2, 3 and 4 and HPRT in the range of 10^4–10^8 copies/μl. Relative mRNA expression ratios (copies of transcript per fg HPRT) were used for subsequent statistical analyses.
Table 1 The histopathologic, clinical and Piwi-like mRNA expression data of STS patients

| Patients’ characteristics | No. of cases | Piwi-like 2 | Piwi-like 3 | Piwi-like 4 |
|--------------------------|--------------|------------|------------|------------|
|                          |              | low | high | low | high | low | high |
| Total                    | 125          | 62 | 63  | 62 | 63  | 62 | 63  |
| Sex                      |              |     |     |     |     |     |     |
| Male                     | 57           | 29 | 28  | 26 | 31  | 27 | 30  |
| Female                   | 68           | 33 | 35  | 36 | 32  | 35 | 33  |
| Histological subtype     |              |     |     |     |     |     |     |
| LS                       | 28           | 10 | 18  | 14 | 14  | 11 | 17  |
| MFH                      | 31           | 12 | 19  | 17 | 14  | 19 | 12  |
| FS                       | 7            | 4  | 3   | 5  | 2   | 3  | 4   |
| RMS                      | 8            | 5  | 3   | 2  | 6   | 5  | 3   |
| LMS                      | 21           | 14 | 7   | 9  | 12  | 9  | 12  |
| NS                       | 12           | 6  | 6   | 4  | 8   | 6  | 6   |
| Syn                      | 10           | 5  | 5   | 7  | 3   | 5  | 5   |
| Other                    | 8            | 6  | 2   | 4  | 4   | 4  | 4   |
| Tumor size\(^1\)         |              |     |     |     |     |     |     |
| T1                       | 29           | 17 | 12  | 10 | 19  | 18 | 11  |
| T2                       | 96           | 45 | 51  | 29 | 67  | 44 | 52  |
| Tumor grade              |              |     |     |     |     |     |     |
| I                        | 19           | 7  | 12  | 7  | 12  | 5  | 14  |
| II                       | 58           | 30 | 28  | 29 | 29  | 31 | 27  |
| III                      | 48           | 25 | 23  | 26 | 22  | 26 | 22  |
| Tumor stage              |              |     |     |     |     |     |     |
| I                        | 16           | 6  | 10  | 7  | 9   | 5  | 11  |
| II                       | 57           | 28 | 29  | 29 | 28  | 29 | 28  |
| III                      | 40           | 21 | 19  | 21 | 19  | 22 | 18  |
| IV                       | 12           | 7  | 5   | 5  | 7   | 6  | 6   |
| Complete resection       |              |     |     |     |     |     |     |
| Radical (R0)             | 87           | 44 | 43  | 44 | 43  | 42 | 45  |
| Not radical (R1)         | 38           | 18 | 20  | 18 | 20  | 18 | 18  |
| Location                 |              |     |     |     |     |     |     |
| Extremities              | 80           | 36 | 44  | 41 | 39  | 41 | 39  |
| Trunk wall               | 12           | 9  | 3   | 8  | 4   | 6  | 6   |
| Head/neck                | 4            | 2  | 2   | 1  | 3   | 4  | 0   |
| Abdomen/retroperitoneum  | 27           | 14 | 13  | 12 | 15  | 11 | 16  |
| Multiple locations       | 2            | 1  | 1   | 0  | 2   | 0  | 2   |
| Patient status           |              |     |     |     |     |     |     |
| Alive                    | 64           | 26 | 38  | 31 | 33  | 30 | 34  |
| Dead                     | 61           | 36 | 25  | 31 | 30  | 32 | 29  |

Abbreviations: No.-number of cases, LS-liposarcoma, MFH-malignant fibrous histiocytoma, FS-fibrosarcoma, RMS-rhabdomyosarcoma, LMS leiomyosarcoma, NS-neuronal sarcoma, Syn-synovial sarcoma.

\(^1\)T1 \(\leq 5\) cm, T2 \(> 5\) cm.

Statistical analyses

Bivariate correlation analyses were performed by Spearman rank correlations (r\(_s\)). Kaplan-Meier and multivariate Cox’s regression analyses were performed to analyze the correlation of Piwi-like 2–4 mRNA expression levels with tumor-specific survival. All statistical analyses were conducted with PASW 18 software (IBM SPSS, Chicago, IL, USA). P-values < 0.05 were considered significant.

Results

Expression of Piwi-like 2–4 mRNAs and their association with clinical, histopathologic and molecular parameters

Piwi-like 2, Piwi-like 3 and Piwi-like 4 mRNA expression levels were measured in tissue samples from 125 STS patients. Cut-off values were set according to the median value for each gene (Piwi-like 2: 7.583 copies/fg HPRT, range: 0.073–532.45), Piwi-like 3: 0.05 copies/fg HPRT, range: 0–528.22) and Piwi-like 4: 1.754 copies/fg HPRT, range: 0–22.89).

In addition we studied the Piwi-like 2, Piwi-like 3 and Piwi-like 4 mRNA expression levels in normal tissue samples adjacent to tumor tissues from 22 out of the 125 STS patients. We detected median mRNA expression levels for Piwi-like 2: 0.5 copies/fg HPRT (range: 0.04–24.84), for Piwi-like 3: 0.0 copies/fg HPRT (range: 0–5), and for Piwi-like 4: 0.28 copies/fg HPRT (range: 0–39.5). The Piwi-like 2, Piwi-like 3 and Piwi-like 4 mRNA expression levels are higher in the tumor tissues compared to those in the normal tumor adjacent tissues. But they are not correlated with each other (Spearman-Rho test) and the median values of their differences are unequal (p < 0.05; Wilcoxon test).

We performed a bivariate linear correlation analysis (Spearman-Rho-test) to examine whether the mRNA expression levels of individual Piwi-like genes were correlated with the mRNA expression levels of the other Piwi-like genes in the tumor tissues. The expression levels of mRNA for Piwi-like 2 and Piwi-like 4 were highly significantly correlated (r\(_s\) = 0.36; p = 0.00003), whereas no significant correlation was detected for either mRNA with Piwi-like 3 in the tumor tissues.

Bivariate linear correlation analysis was also performed to analyze the relationship of Piwi-like mRNA expression with clinical factors. The mRNA levels of all Piwi-like genes studied were positively correlated to tumor size (Piwi-like 2: r\(_s\) = 0.21; p = 0.018; Piwi-like 3: r\(_s\) = 0.20; p = 0.024; and Piwi-like 4: r\(_s\) = 0.18; p = 0.041), Next, we investigated the correlation of Piwi-like 2–4 mRNAs with other stem cell-associated genes (Nanog, Oct3/4 and survivin). Piwi-like 2 mRNA expression was inversely correlated with transcript levels of Oct3/4 and Nanog in our STS cohort (r\(_s\) = −0.31; p = 0.002 and r\(_s\) = −0.23; p = 0.029, respectively) but was positively correlated with the splice variant 2B of the survivin gene (r\(_s\) =
0.20; p = 0.048). Piwi-like 3 mRNA expression was also negatively correlated with Nanog transcript levels (r_s = −0.24; p = 0.02). Piwi-like 4 mRNA levels were negatively associated with the expression of the splice variant Delta 3 of the survivin gene (r_s = −0.18; p = 0.047; Additional file 2: Table S1).

Association of the Piwi-like 2–4 mRNA expression level with tumor-specific survival for all STS patients

Multivariate Cox’s regression analyses (adjusted for clinical parameters including resection type, tumor stage, tumor location and tumor type) were performed to study the effect of the mRNA expression level of the three Piwi-like genes on tumor-specific survival. For Piwi-like 2, low mRNA expression was significantly associated with a worse prognosis (RR = 1.87, 95% CI: 1.06–3.32; p = 0.032). Low Piwi-like 4 mRNA expression also correlated significantly with decreased tumor-specific survival (RR = 1.82, 95% CI: 1.03–3.21; p = 0.039). Piwi-like 3 expression was not significantly associated with the prognosis of STS patients (RR = 1.33, 95% CI: 0.75–2.3; p = 0.329).

Next, we studied the combined expression of the Piwi-like 2 – 4 and their correlation with tumor-specific survival. Patients expressing low levels of either Piwi-like 2/ Piwi-like 3 or of Piwi-like 2/Piwi-like 4 had a significantly increased risk of tumor-related death compared with patients with a high expression level of the Piwi-like mRNAs (RR = 2.10, 95% CI: 1.01–4.40; p = 0.048 and RR = 2.58, 95% CI: 1.26–5.29; p = 0.01, respectively; Table 2).

Association of the Piwi-like 2–4 mRNA expression level with tumor-specific survival in a sex-specific manner for STS patients

We performed separate analyses of the association of Piwi-like 2–4 expression in male and female patients (Additional file 3: Figure S2). Female patients with a low level of Piwi-like 4 mRNA expression in their tumors had a 3.53-fold significantly increased risk of tumor-related death (95% CI: 1.56–8.0; p = 0.002). Furthermore, in females, a trend for increased risk of tumor-related death was seen in patients with low levels of Piwi-like 2 expression (RR = 1.88, 95% CI: 0.87–4.07). However, this result was not statistically significant (p = 0.106). Male patients with low levels of Piwi-like 2 or Piwi-like 3 had a 2.76-fold and a 2.59-fold increased risk of tumor-related death. However, this result was also not statistically significant (Table 3).

Finally, we analyzed the combined expression of the Piwi-like 2–4 genes and their correlation with tumor-specific survival in a sex-specific manner and observed that different expression profiles were significantly correlated with survival in male and female patients. For male patients, decreased expression of Piwi-like 2/ Piwi-like 3 was correlated with a 5.9-fold increase in the risk of tumor-related death (95% CI: 1.33–26.23; p = 0.02). In female patients, decreased Piwi-like 2/ Piwi-like 4 expression was correlated with a 5.23-fold increase in the risk of tumor-related death (95% CI: 1.67–16.32; p = 0.004; Table 3 Figure 1). The results revealed that the combined expression of Piwi-like genes is correlated with tumor-specific survival in a sex-specific manner.

Table 2 Multivariate Cox’s regression analyses on the impact of Piwi-like 2, -3 and -4 mRNA expression on STS patients’ survival

| Piwi-like 2 | Piwi-like 3 | Piwi-like 4 |
|-------------|-------------|-------------|
| Relative risk (RR) | 1.87 | 1.33 | 1.82 |
| 95% CI | 1.06–3.32 | 0.75–2.3 | 1.03–3.21 |
| p-value | **0.032** | 0.329 | **0.039** |
| n low | 62 | 62 | 62 |
| n high | 63 | 63 | 63 |

| Combination | Combination |
|-------------|-------------|
| Piwi-like 2 and | Piwi-like 2, -3 and -4 |
| Relative risk (RR) | 2.58 | 4.01 |
| 95% CI | 1.26–5.29 | 1.61–10.01 |
| p-value | **0.01** | **0.003** |
| n all low | 39 | 21 |
| n one gene elevated | 46 | 43 |
| n two genes elevated | 27 | 37 |
| n all genes elevated | 40 | 24 |

Significant p-values and relative risks are marked in bold face (p-values < 0.05 were considered significant).

Discussion

Previously we found that elevated levels of Piwi-like 1 in STS and high as well as low levels of Piwi-like 1 in pancreatic adenocarcinomas were significantly associated with worse patient’s survival. [18,19]. In our study, the levels of Piwi-like 2–4 mRNA in STS were analyzed and correlated with each other, with clinical and histopathologic parameters and with tumor-specific survival. We found a significant correlation between tumor-specific survival and Piwi-like 2 and Piwi-like 4 mRNA expression levels, but not with Piwi-like 3 gene expression. There were correlations between the mRNA expressions of the Piwi-like genes and other stem cell-associated genes, such as Nanog, Oct3/4 and survivin. Surprisingly, a low expression of Piwi-like 2 or Piwi-like 3 was significantly correlated with a high expression level of Nanog and Piwi-like-2 in addition with an increased transcript level of Oct3/4, but with a decreased level of the survivin 2B splice variant. Furthermore, low
Piwi-like 4 mRNA expression was correlated with a high level of the survivin Delta 3 splice variant. However, the survivin 2B splice variant is considered to be a pro-apoptotic protein, whereas the survivin Delta 3 splice variant has an anti-apoptotic function [22]. PIWIL2 protein may inhibit apoptosis via activation of Stat3/Bcl-X signaling [20].

A somewhat surprising result of our study was that a low mRNA expression level of all three Piwi-like genes was correlated with a poor prognosis, although higher protein levels have been reported to be associated with a poor survival [8-11]. There are several possible explanations for this phenomenon. Low mRNA transcription levels may characterize more aggressive tumors. For example, low mRNA levels for the oncogenes mdm2 and c-myc have been correlated with a poor prognosis of patients with STS and squamous carcinomas of the tongue, respectively [23,24]. Another possibility is that there could be a negative autoregulatory feedback loop between protein and RNA levels in Piwi-like genes. Such a feedback loop has been described for other genes, for example for Snail1. Snail1 is a transcriptional repressor that binds to its own promoter

Figure 1 Multivariate Cox's regression analyses: A. Combined expression of Piwi-like 2 and 3 in male STS patients and B. combined Piwi-like 2 and −4 expression in female STS patients and their correlation with tumor-specific survival.
and controls its expression [25]. Analogously, one may speculate that Piwi-like proteins, as the main effector component of the RNA-induced silencing complex (RISC), may regulate their own expression by post-transcriptional gene silencing via an unknown small RNA binding partner. Furthermore, different alternative transcripts leading to shortened protein products of PIWIL2 have been described recently [12]. The predominant form, PL2L60, is mainly expressed in tumor cells and promotes cell survival and proliferation of a human breast cancer cell line (MDA-MB-231), possibly by the upregulation of Bcl2 and Stat3. Because our primers targeted the 5’ end of the different mRNA transcripts for a better discrimination between the homologous genes, we were not able to measure the shortened transcript. Further studies are needed to determine if shortened alternative mRNA transcripts and protein isoforms for Piwi-like 3 and Piwi-like 4 also exist. Additionally, shorter transcripts and/or their protein products could decrease the levels of their full-length transcripts.

Piwi-like proteins bind exclusively to Piwi-interacting RNA (piRNA), a class of small RNAs that differ structurally from the classic siRNA and miRNA in several ways, including length (24–30 nt instead of 18–23 nt), the carriage of a 2’O-methyl modification at the 3’ end and their low conservation among even closely related species [26–28]. piRNAs are transcribed from a limited set of clusters located in pericentromeric or telomeric heterochromatic regions by an as yet unknown mechanism [29]. Their maturation is involved in a set of degradation steps involving transposon transcripts. Uncontrolled transposon activation in Piwi gene mutants has been observed in Drosophila and in mice [30,31], which points to a role of Piwi-like genes in transposon control during spermatogenesis. However, the mechanisms and consequences of Piwi-like gene re-initiation in tumors are not yet fully understood. Given our observation that a low mRNA expression of Piwi-like genes is correlated with increased tumor size and worse survival, one may speculate that a decrease in the mRNA of Piwi-like genes can derepress transposon activity and enhance tumor cell selection to a more proliferative, active and aggressive phenotype.

PIWI proteins appear to have sex-specific functions, especially in vertebrate male germ cell maturation [30–32]. We were able to show for the first time a sex-specific effect of Piwi-like gene expression on the survival of tumor patients. The presence of androgen and estrogen receptors has been reported in soft tissue sarcomas [33]. An in-silico search for binding sites for sex-steroid receptors in the putative promoter regions of the Piwi-like genes showed an androgen receptor binding site in the Piwi-like 2 gene promoter (unpublished results). However, the transcriptional activation of the Piwi-like genes may also be initiated indirectly by transcription factors regulated by sex-steroid binding factors. This question should be addressed in further studies.

Table 3 Sex-specific association between Piwi-like 2–4 mRNA expression and STS patient survival

|                | Female patients | Male patients |
|----------------|-----------------|--------------|
|                | Piwi-like 2     | Piwi-like 3   | Piwi-like 4 |
|                | Piwi-like 2     | Piwi-like 3   | Piwi-like 4 |
| Relative risk (RR) | 1.88           | 1.13         | **3.53**   |
| 95% CI         | 0.87–4.07      | 0.51–2.52    | 1.56–8.00  |
| p-value        | 0.106          | 0.757        | **0.002**  |
| n low          | 34             | 34           | 34         |
| n high         | 34             | 34           | 34         |
|                | Combination Piwi-like 2 and –4 | Relative risk (RR) | 95% CI | p-value |
|                | 15             | **5.90**     | 1.33–26.23 | 0.02 |
| n all low      | 22             | **5.23**     | 1.67–16.32 | 0.004 |
| n one gene elevated | 24         | **4.47**     | 1.47–13.61 | 0.008 |
| n two genes elevated | 22         | reference    | reference | reference |

Significant p-values and relative risks are marked in bold face (p-values < 0.05 were considered significant).

In conclusion, a correlation of Piwi-like 2–4 mRNA expression with tumor size and of Piwi-like 2 and Piwi-like 4 transcript levels with tumor-related death was found for STS patients. Additionally, there was a sex-specific association of the combination of low mRNA transcript levels for Piwi-like 2/3 and Piwi-like 2/4 with tumor-specific survival in male and in female STS patients, respectively. A low mRNA expression level for Piwi-like 2–4 genes defines a significantly increased risk for tumor-related death and may have potential as a predictor of survival for both all STS patients and in a sex-specific manner.
Competing interests

The authors report no potential conflicts of interest.

Authors' contributions

TG designed the study, analyzed the data and drafted the manuscript. FK performed experimental procedures, carried out molecular biological studies and analyzed the data, MKA and MBB aided in study design, analyzed the data and reviewed the manuscript. PW treated the patients, collected tumor/normal tissues and clinical data and participated in the study design, CL and SG performed and reviewed statistical analysis, HI/II performed histopathological evaluation and re-evaluation of sarcoma tissues, SW carried out molecular biological studies and analyzed the data, HT designed the study, analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

H.T.’s work was supported by the Deutsche Krebshilfe (No. 107590) and was associated with the German Sarcoma Study Group K.O.S.A.R. T.G.’s work was supported by a grant of the Wilhelm-Roux program (FKZ 241/15). We would like to thank the American Journal Experts for language editing of our manuscript.

Author details

1. Center for Reproductive Medicine and Andrology, Martin-Luther-University Halle-Wittenberg, Halle, Germany. 2. Department of Oral and Maxillofacial Plastic Surgery, Martin-Luther-University Halle-Wittenberg, Halle, Germany. 3. Institute of Medical Biometry and Informatics, Martin-Luther-University Halle-Wittenberg, Halle, Germany. 4. Department of Radiotherapy, Martin-Luther-University Halle-Wittenberg, Halle, Germany. 5. Institute of Pathology, Martin-Luther-University Halle-Wittenberg, Halle, Germany. 6. Institute of Transfusion Medicine, Martin-Luther-University Halle-Wittenberg, Halle, Germany. 7. Institute of Pathology, Martin-Luther-University Halle-Wittenberg, Halle, Germany. 8. Div. Molecular Urology, FAU Erlangen-Nürnberg, University Clinic of Urology, Erlangen, Germany. 9. Ulrich-Fiebig-Center for Molecular Medicine, FAU Erlangen-Nürnberg, Erlangen, Germany. 10. Department of General and Visceral Surgery, Diakoniekrankenhaus Halle, Halle, Germany. 11. Klinik für Urologie und NFZ, FAU Erlangen, Glücksstr. 6, D-91054 Erlangen, Germany.

Received: 22 November 2011 Accepted: 15 May 2012 Published: 29 June 2012

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