# Expression of 3q oncogene *SEC62* in atypical fibroxanthoma-immunohistochemical analysis of 41 cases and correlation with clinical, viral and histopathologic features

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**Abstract.** Atypical fibroxanthoma (AFX) is a rare mesenchymal tumor with predominance in older male patients located mainly in chronically UV-exposed skin. Differentiation from clinically more aggressive pleomorphic dermal sarcoma (PDS) is still under debate and immunohistochemical markers are not available yet. An immunohistochemical study, including 41 cases of AFX was conducted to investigate the expression of 3q encoded oncogene *SEC62* in AFX and determine the associations with histomorphologic, clinical and viral parameters. Our cohort displayed a mean of 79.9 years at the onset of the disease. In total, 90.2% (37/41) AFXs were located in the head and neck area, whereas, four were located at the extremities (9.7%). Tumor diameter ranged between 0.06 and 40 $cm^2$ with a mean of 5.7 $cm^2$. *SEC62* expression was markedly increased in lesional tissue compared with the adjacent healthy squamous epithelium. We found significantly higher expression of *SEC62* in cases of AFX with tumor necrosis. Tendency of higher Sec62-IRS-scores were found for tumors with higher Clark levels and a tumor size >5 $cm^2$. Sec62 is involved in endoplasmic reticulum stress tolerance and cell migration, and has been identified as a novel prognostic marker for non-small cell lung cancer as well as head and neck squamous cell carcinoma. For the first time, to the best of our knowledge, we suggest a role of 3q oncogene *SEC62* in AFX and discuss a potential prognostic relevance in cases of disputable AFX with unfavorable histomorphologic features and may initiate a discussion on Sec62 serving as discriminating marker between AFX and PDS.

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## Introduction

Atypical fibroxanthoma (AFX) is a comparably rare dermal neoplasm with only 3,000 cases reported in the literature so far (1). This tumor predominantly develops at sun-exposed areas of the human body with emphasis on the head and neck region, and typically affects more men than women with a median age above 60 years (1,2). While AFX can often show a large extension in the superficial skin layers, an invasion of deeper structures e.g., blood or lymph vessels, subcutaneous muscles or peripheral nerves is usually not found resulting in a classification as benign or semi-malignant tumor (3,4). Hence, the prognosis of AFX patients is excellent with a median 20-year disease-specific survival rate of 97.8% (1). After its first description in 1963, much effort was spent to better understand the tumorigenesis and molecular biology of this tumor entity including immunohistochemical analyses, electron microscopy, comparative genomic hybridization and next generation sequencing (5,6). However, the pathogenesis of AFX is still unclear with keratinocytes, fibroblasts and myofibroblasts having been discussed as potential cells of origin (7,8). Since this tumor shows a highly heterogeneous histological structure with tumor cells ranging from spindle and epithelioid to multinucleated cells and a variably structured extracellular matrix, the histological diagnosis is challenging with undifferentiated pleomorphic sarcoma, malignant fibrous histiocytoma, dedifferentiated squamous cell carcinoma, dermatofibrosarcoma protuberance and leiomyosarcoma as differential diagnoses (1,9). Immunohistochemical markers that can help to differentiate AFX from other tumor entities are CD99, S-100, CD34, cytokeratin, desmin, CD10, vimentin, HMB-45, CD68 and p63 (8-12). However, a disease-specific marker as well as a prognostic marker indicating a higher risk of recurrence or distant metastasis is still missing (1,3,4). In 2010 an association to Merkel Cell Polyomavirus was detected in 17% of all AFXs examined (13). Importance of this finding has to be further elucidated.

The *SEC62* gene located at chromosomal region 3q26.2 encodes for a transmembrane protein of the endoplasmic reticulum (ER) that forms a heterotrimeric complex with the protein translocation pore and intracellular calcium channel Sec61 as well as Sec63 (14,15). Under physiological conditions, Sec62 is involved in the posttranslational transport of short secretory and transmembrane precursor proteins, possibly through its direct interaction with Sec61 and the ribosome (16-18). Apart from protein transport, Sec62 was shown to influence the passive calcium efflux through the Sec61 channel into the cytosol in an inhibitory manner (19-22). An amplification of the *SEC62* encoding region 3q26.2 as well as an overexpression of the *SEC62* gene was observed in various cancer entities including head and neck cancer (23,24), prostate cancer (25), esophageal cancer (26), cervical cancer (27,28), ovarian cancer (29) and non-small cell lung cancer (30). For NSCLC and HNSCC, high *SEC62* expression level was a predictor of poor clinical outcome and significantly correlated with a positive lymph node status (31-33). In hepatocellular cancer, high *SEC62* expression levels were correlated with a higher risk of recurrence after surgical treatment (34). Beneath its role as a prognostic biomarker, Sec62 was shown to influence tumor cell biology by stimulating cancer cell migration, invasion and enabling tumor cells to recover from ER stress by a mechanism called 'recovER-phagy' (21,35-39). These effects can explain how tumor cells profit from an increased *SEC62* expression level and might be responsible for the poor prognosis of *SEC62* overexpressing tumors. Based on the finding that the stimulation of cancer cell migration by Sec62 is probably mediated through its influence on the calcium homeostasis at the ER, the calmodulin antagonist trifluoperazine (TFP) could be identified as a potent agent to antagonize the calcium effect of Sec62 and thereby inhibiting Sec62 mediated cancer cell migration (21). Hence, TFP represents a promising agent for an antimetastatic therapy in *SEC62* overexpressing tumors.

As for AFX, there exist neither reliable immunohistochemical markers enabling discrimination from other related sarcomatoid tumors nor prognostic biomarkers indicating a higher risk of recurrence or distant metastasis, we investigated in our study the expression of 3q oncogene *SEC62* in 41 AFX cases and correlated the *SEC62* expression level with the patients' clinical and viral data and the pathological characteristics of the tumors.

## Materials and methods

*General*. Investigations were performed after approval by a local Human Investigations Committee, approval no. 281/10 (Ethikkomission der Ärztekammer des Saarlandes).

*Patient characteristics and tissue samples*. AFXs were retrieved from the histopathology archives of the department of dermatology. A period from 2006 to 2016 was investigated. Inclusion criteria for the study were availability of slides and blocks as well as tumors treated surgically by excision with curative intention. A total of 41 AFXs of 40 patients were investigated in this study. The following clinical and histopathologic features were evaluated: Sex, age and size, mitotic count, presence of necrosis, ulceration, vascular invasion as well as invasion depth and Clark level. Follow-up information was obtained from hospital medical records of the referring clinicians. Details of clinicopathological characteristics are summarized in Table I. Detailed histopathologic data is given in Table II.

*Immunohistochemical analysis*. FFPE tissue sections were obtained and used for immunohistochemical staining of Sec62. After omitting the first three 10-$\mu$m sections, consecutive 4-$\mu$m sections were obtained using a Leica RM 2235 rotary microtome (Leica Microsystems, Wetzlar, Germany), transferred onto Superfrost Ultra Plus microscope slides (Menzel-Gläser, Braunschweig, Germany) and dried in an incubator at 37˚C overnight. Upon deparaffinization, heat-induced epitope retrieval was performed by incubation in 10 mM citrate buffer (pH 6.0) at 95˚C for 30 min. Unspecific protein binding sites were blocked with 3% BSA (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) in PBS for 30 min at room temperature. Subsequently, primary antibody incubation was performed using an affinity-purified polyclonal rabbit anti-peptide antibody directed against the C terminus of human Sec62 (self-made). For each staining series, a specimen taken from a subcutaneously grown tumor in mice after local injection of UM-SCC1 cells (*SEC62* overexpressing cell line) was used as positive control as well as negative controls by omitting the primary antibody. Visualization was performed using the REAL™ detection system Alkaline Phosphatase (Dako Agilent Technologies, Glostrup, Denmark), according to the manufacturer's instructions, and the slides were counterstained with hematoxylin (Dako Agilent Technologies). Sec62-immunoreactivity was evaluated using an immunoreactive score (IRS) according to Remmele and Stegner (40) with values ranging from 0 to 12. All immunohistochemical stainings were valued by three experienced examiners including one dermatopathologist and the mean values of the three scorings were used for statistical analysis. 34/41 cases were available for immunohistochemistry with sec62.

Specific *detection of mitoses* was performed using phosphohistone H3 (pHH3; polyclonal antibody, Cell Marque® no. 369A) at a dilution of 1:100 (pre-treatment for 30 min in a steamer) in accordance with the manufacturer's protocol. Mitotic figures labeled with pHH3 were twice counted in 10 high-power-fields within the 'hot spot' of the tumour and mean of mitoses was calculated.

*MCPyV-DNA PCR*. MCPyV-DNA-specific PCR was performed for all FFPE tissue specimens (n=41). DNA was extracted from the FFPE tissue samples using a QIAamp DNA FFPE Tissue kit (Qiagen N.V., Hilden, Germany) according to the manufacturer's instructions. MCPyV-DNA-PCR was performed with the LightCycler 2.0 instrument (Roche Diagnostics GmbH, Mannheim, Germany) using MCPyV-specific primers LT3F (5'-ttgtctcgccagcattgtag-3') and LT3R (5'-atataggggcctcgtcaacc-3') described by Feng *et al* (41). Cycling conditions were 94˚C for 3 min, followed by 45 PCR cycles with denaturation at 94˚C for 45 sec, annealing at 58˚C for 30 sec, elongation at 72˚C for 45 sec and finished by a last elongation at 72˚C for 15 min. After amplification, the PCR products (10 $\mu$l) were separated on 2% agarose gels and visualized by ethidium bromide staining. Glyceraldehyde 3-phosphate dehydrogenase

Table I. Clinical data of all patients enrolled in the present study.

| Patient no. | Age at time of diagnosis | Sex | Localization | Tumor diameter | Relapse | Distant metastases | Death |
|---|---|---|---|---|---|---|---|
| 1 | 63 | M | Head | 4.00 | No | No | No |
| 2 | 78 | M | Head | N.A. | No | No | No |
| 3 | 83 | M | Upper extremity | 1.00 | No | No | Yes |
| 4 | 84 | F | Head | 1.00 | No | No | No |
| 5 | 81 | M | Face | 0.06 | No | No | No |
| 6 | 77 | M | Head | 0.75 | No | No | No |
| 7 | 85 | M | Head | 4.00 | No | No | No |
| 8 | 81 | M | Head | 0.49 | No | No | Yes |
| 9 | 65 | M | Head | N.A. | No | No | No |
| 10 | 81 | M | Head | 1.00 | No | No | No |
| 11 | 83 | F | Head | N.A. | No | No | No |
| 12 | 78 | M | Head | 1.00 | No | No | Yes |
| 13 | 65 | M | Head | 1.65 | No | No | No |
| 14 | 81 | M | Head | N.A. | No | No | No |
| 15 | 81 | F | Head | 3.00 | No | No | No |
| 16 | 78 | M | Upper extremity | 4.00 | Yes | No | No |
| 17 | 79 | M | Head | 0.25 | No | No | No |
| 18 | 79 | M | Head | 2.50 | No | No | Yes |
| 19 | 84 | M | Head | N.A. | No | No | No |
| 20 | 72 | F | Lower extremity | 1.00 | No | No | No |
| 21 | 87 | M | Head | N.A. | No | No | No |
| 22 | 90 | F | Head | 4.00 | Yes | No | No |
| 23 | 92 | F | Head | 5.00 | No | No | No |
| 24 | 79 | M | Head | N.A. | No | No | No |
| 25 | 87 | M | Head | 40.00 | No | No | No |
| 26 | 80 | F | Face | 9.00 | No | No | No |
| 27 | 88 | M | Head | 25.00 | Yes | No | Yes |
| 28 | 79 | M | Head | 9.00 | No | No | No |
| 29 | 78 | M | Head | N.A. | No | No | No |
| 30 | 76 | M | Head | 5.75 | No | No | No |
| 31 | 80 | M | Head | 22.00 | No | No | No |
| 32 | 89 | M | Head | 9.00 | No | No | No |
| 33 | 87 | M | Head | 3.00 | No | No | No |
| 34 | 83 | M | Head | 9.00 | No | No | No |
| 35 | 84 | M | Head | N.A. | No | No | Yes |
| 36 | 78 | M | Head | 5.00 | No | No | No |
| 37 | 73 | M | Face | 0.32 | No | No | No |
| 38 | 72 | M | Lower extremity | 2.70 | No | No | No |
| 39 | 62 | M | Head | 1.00 | No | No | No |
| 40 | 88 | M | Head | N.A. | No | No | No |
| 41 | 89 | F | Face | 1.00 | No | No | No |

M, male; F, female; N.A., not applicable.

(GAPDH) PCR served as an internal control and was performed in parallel for each sample as described previously by Sperling *et al* (42).

*Statistical analysis*. Beside descriptive statistical analyses (frequencies, mean and standard deviation) the comparison of groups was performed with the Mann-Whitney U test resp. Kruskal-Wallis-Test. The analyses were executed with SPSS v. 17.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Table II. Histomorphologic data of all atypical fibroxanthomas of the present study.

| Patient no. | Vertical tumor depth, mm | Clark level | Necrosis | Ulceration | Mitotic count | Vascular invasion | MCPyV DNA | *SEC62* Immunoscore |
|---|---|---|---|---|---|---|---|---|
| 1 | 8.35 | 5 | Yes | Yes | 8.70 | No | + | 9.0 |
| 2 | 2.68 | 3 | No | Yes | 3.55 | No | - | 2.6 |
| 3 | 2.20 | 3 | No | No | 2.40 | No | + | 6.0 |
| 4 | 2.90 | 4 | No | Yes | 4.40 | No | - | 8.3 |
| 5 | 2.20 | 5 | No | No | 1.50 | No | - | N.A. |
| 6 | 2.40 | 4 | No | No | 2.70 | No | - | N.A |
| 7 | 2.16 | 3 | No | Yes | 4.60 | No | - | 5.6 |
| 8 | 5.70 | 2 | No | Yes | 5.90 | No | - | 4.0 |
| 9 | 2.16 | 3 | No | No | 4.00 | No | - | 4.3 |
| 10 | 7.20 | 5 | Yes | Yes | 4.80 | No | - | 1.0 |
| 11 | 8.00 | 5 | No | Yes | N.A. | No | - | 6.3 |
| 12 | 3.10 | 4 | No | No | 4.20 | No | - | 11.0 |
| 13 | 6.90 | 4 | No | No | 4.80 | No | - | 9.3 |
| 14 | 4.08 | 5 | No | Yes | 5.70 | No | - | 10.0 |
| 15 | 7.50 | 5 | No | Yes | 7.50 | No | - | 8.0 |
| 16 | 5.80 | 5 | No | Yes | 5.70 | No | - | 8.0 |
| 17 | 1.29 | 4 | No | No | 2.40 | No | - | 8.6 |
| 18 | 4.80 | 4 | Yes | Yes | 1.10 | No | - | 12.0 |
| 19 | 14.00 | 2 | No | Yes | 8.90 | No | - | 10.0 |
| 20 | 2.40 | 2 | No | Yes | 3.70 | No | - | 6.6 |
| 21 | 6.52 | 5 | No | Yes | 1.00 | No | - | 3.6 |
| 22 | 5.28 | 5 | No | Yes | 5.70 | No | - | 7.3 |
| 23 | 2.60 | 4 | No | Yes | 3.80 | No | + | 8.0 |
| 24 | 4.08 | 4 | Yes | Yes | 5.60 | No | + | 11.0 |
| 25 | 40.00 | 5 | Yes | Yes | N.A. | No | - | 10.0 |
| 26 | 4.80 | 5 | No | Yes | 50.40 | No | - | 7.0 |
| 27 | 2.90 | 4 | No | Yes | 5.10 | No | - | 9.0 |
| 28 | 4.08 | 4 | No | No | 6.10 | No | - | 6.0 |
| 29 | 2.68 | 3 | No | Yes | 3.50 | No | - | 6.6 |
| 30 | 9.50 | 4 | No | No | 5.60 | No | - | 11.0 |
| 31 | 5.80 | 4 | No | Yes | 3.70 | No | - | 9.0 |
| 32 | 7.20 | 5 | No | Yes | 8.40 | No | - | 6.0 |
| 33 | 7.68 | 5 | No | No | 7.00 | No | - | 11.0 |
| 34 | 4.27 | 3 | Yes | Yes | 10.20 | No | - | 12.0 |
| 35 | 4.17 | 5 | No | Yes | 7.90 | No | - | 6.6 |
| 36 | 6.50 | 5 | No | Yes | 4.90 | No | - | 6.6 |
| 37 | 4.80 | 4 | No | Yes | 3.30 | No | - | N.A. |
| 38 | 1.90 | 3 | No | No | 3.60 | No | - | N.A. |
| 39 | 4.30 | 5 | No | No | 2.50 | No | - | N.A. |
| 40 | 6.40 | 5 | No | Yes | 3.80 | No | - | N.A. |
| 41 | 2.00 | 3 | No | No | 4.80 | No | - | N.A. |

N.A., not applicable.

## Results

*Clinical and pathological characteristics of patients*. Details of clinicopathological characteristics are summarized in Table I. Detailed histopathologic data is given in Table II. 33 male and 8 female patients were included in this study. Age at time of diagnosis ranged from 62 to 92 years, with a mean of 79.9 years. Clinical information on exact localization of tumors was available in all tumors. 90.2% (37/41) of tumors were located in the head and neck area, while 4 where located at the extremities (9.7%). Tumor diameter ranged from 0,06 to 40 $cm^2$ with a mean of 5.7 $cm^2$.

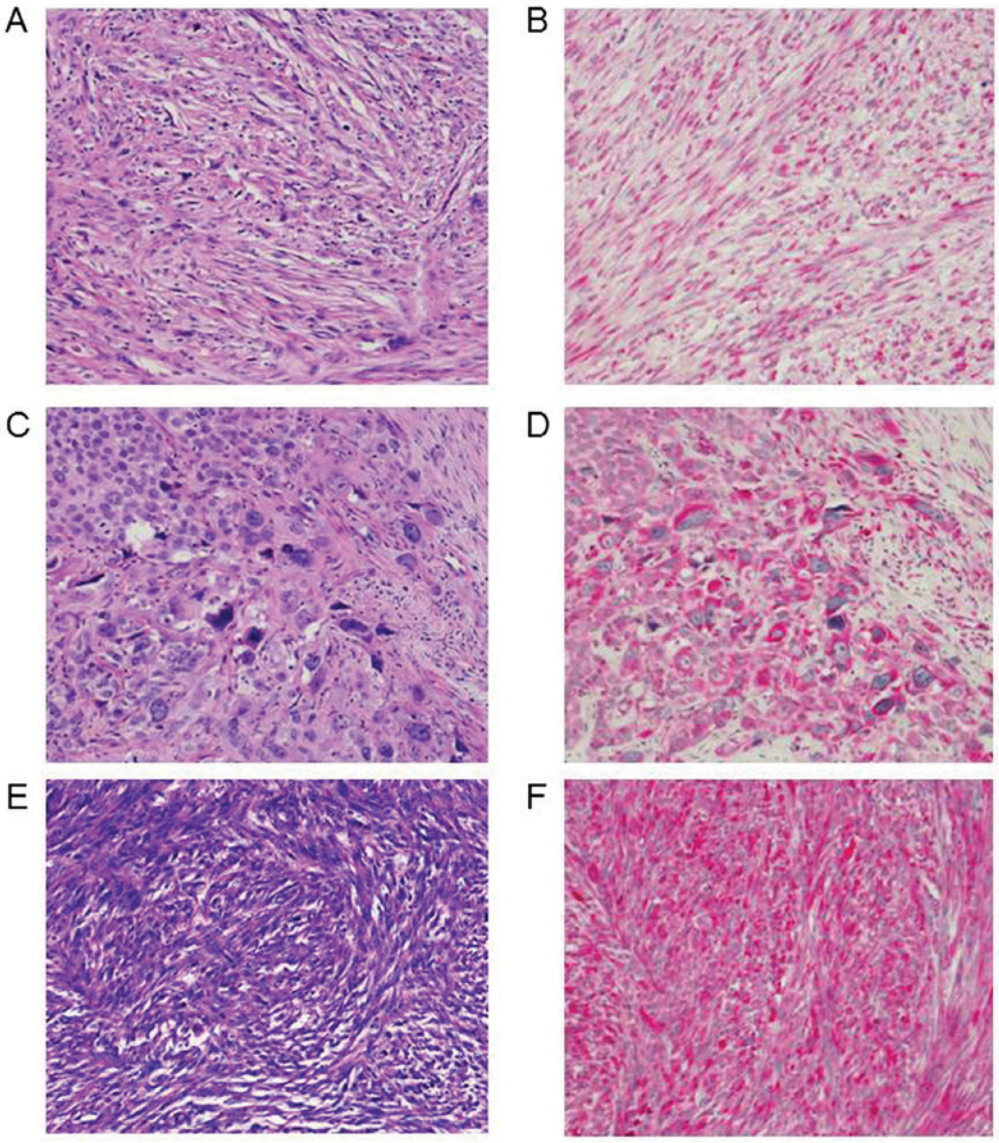


Figure 1. Differential cytoplasmatic expression of 3q oncogene *SEC62* in AFX. Left column see hematoxylin and eosin-stain, original magnification, x200. Right column see Sec62-stain. Original magnification, x200. (A) AFX, patient no. 21: Mixed type with high nuclear pleomorphism and partly storiform tumor growth of mainly spindle-shaped cells. (B) Patient no. 21: Sec62-IRS 3,6. (C) AFX, patient no. 20: Pleomorphic type tumor with bizarre tumor cells and highly atypical mitotic figures. Please note areas with epithelioid differentiation. (D) Patient no. 20: Sec62-IRS 6,6. (E) AFX, patient no. 15: Storiform type of tumor with predominantly spindle-shaped cells in a regular growth. (F) Patient no. 15: Sec62-IRS 8. AFX, atypical fibroxanthoma.

One female patient developed 2 separate tumors, when she was 90 years old and the second when she was 92 of age. Relapse of AFXs was seen in three patients (7.3%), distant metastases did not occur in any patient of this cohort. 6/40 (14.6%) patients died within observation period. Due to low number of cases we did not differentiate between the several morphologic variants of AFX, that have been described (spindle-cell nonpleomorphic AFX, clear-cell AFX, pigmented, myxoid, osteoclast-like giant cell rich AFX keloidal and granular cell AFX)[43]. Invasion depth of all tumors ranged from 1.29 to 40 mm with a mean of 5.6 mm. In analogy to Clark Level (CL) in malignant melanoma (CL I-melanoma in situ, CL II-infiltration of the upper part of the papillary dermis, CL III-expansion of melanoma cells into the papillary dermis and upper reticular dermis, CL IV-infiltration of the reticular dermis and CL V-infiltration of subcutis[44]) we analyzed invasion of anatomic levels of the skin: CL I is not applicable per definitionem as AFXs are primary dermal tumors. CL II was seen in 7% (3/41), CL III was observed in 19% (8/41). Thirteen cases showed CL IV (31%) and 41% (17/41) of the cases displayed extension to subcutaneous tissue. Tumor necrosis was observed in 14.3% (6/41), ulceration of the overlying epidermis was seen in 69% (28/40). Vascular invasion was seen in none of the cases of this cohort. Merkel Cell Polyomavirus (MCPyV)-DNA was detected in 4 cases (9.7%). As a trend, three of these cases were male with ulcerated tumors and one patient was female. One of these 4 patients died within observation period. A mean of 5.9 mitoses could be detected in 10 hotspot areas of every tumor.

*Expression analysis of SEC62 oncogene*. Immunoscore of 3q oncogene *SEC62* could be investigated in 34 cases of all AFXs (Fig. 1). In 100% (34/34) of the samples cytoplasmatic expression of 3q oncogene *SEC62* within the mesenchymal

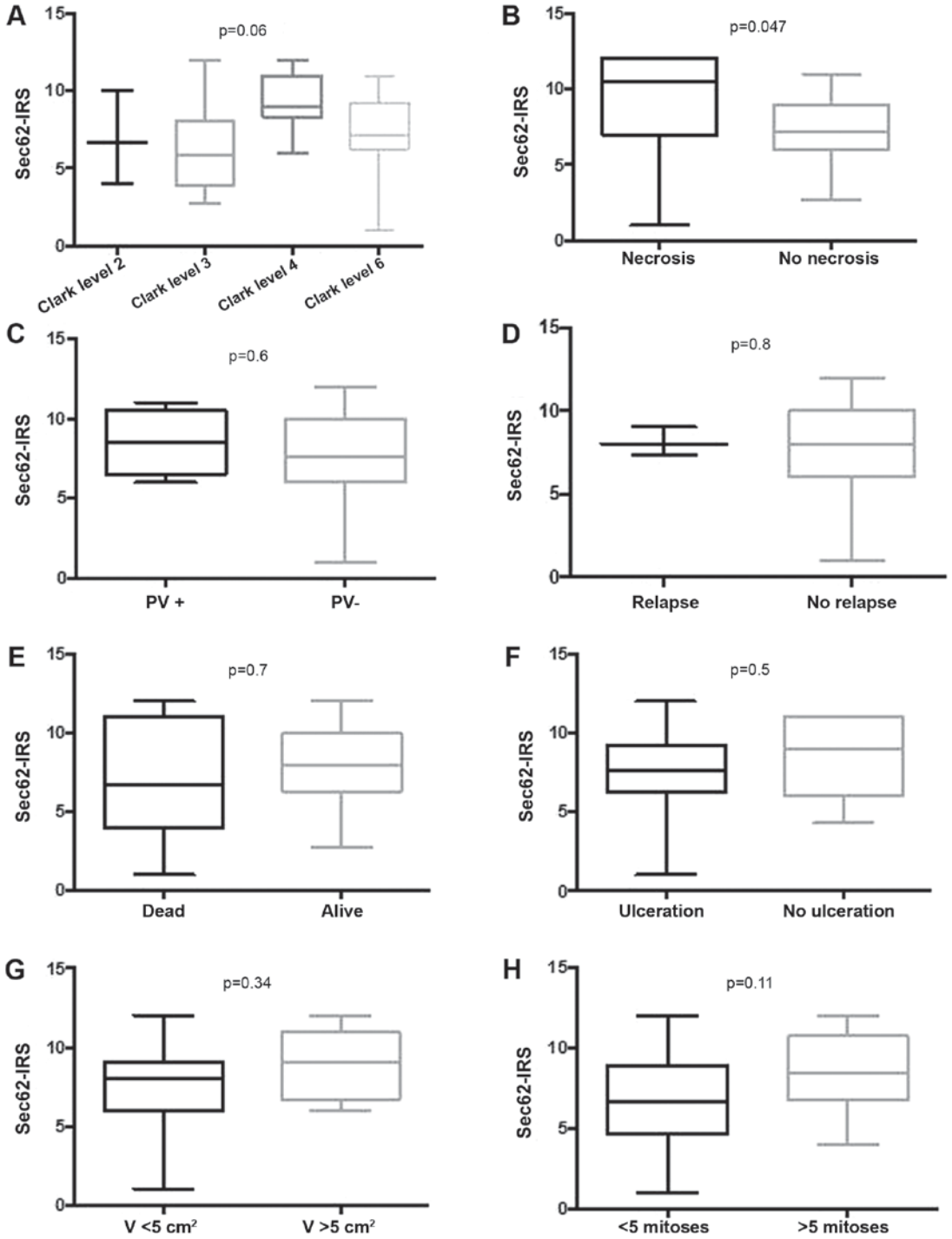


Figure 2. Correlation of Sec62-IRS with clinical and histopathological features. (A) Correlation of Sec62-IRS with tumor stage according to the Clark level. (B) Correlation of Sec62-IRS with the presence of intratumoral necrotic areas. (C) Correlation of Sec62-IRS with the presence of polyomavirus DNA in lesional tissue. (D) Correlation of Sec62-IRS with a relapse of the disease. (E) Correlation of Sec62-IRS with the non-disease-specific survival of the patients. (F) Correlation of Sec62 with an ulcerous growth pattern of the tumor. (G) Correlation of Sec62-IRS with the volume of the tumor. (H) Correlation of Sec62-IRS with the frequency of mitoses. Sec62-IRS values are shown using box and whisker blots. Each box represents the range from the first quartile to the third quartile. The median is indicated by a line. The whiskers outside the boxes represent the ranges from the minimum to the maximum value of each group. IRS, immunoreactive score; PV, Polyomavirus.

tumor cells was seen. Mean score was 7.7 with a minimum of 1 and a maximum score of 12. Exemplary cases of distinct Sec62-IRS-scores are shown in Fig. 1. Intriguingly, we found significantly higher expression of *SEC62* in cases of AFX with tumor necrosis (Fig. 2G), while there was no statistically relevant dependency on invasion depth, ulceration, relapse of tumor, distant metastases or death due to tumor (Fig. 2). Hence, tendency of higher *SEC62*-IRS-scores were found for tumors with higher Clark levels and a tumor size greater 5 $cm^2$, although reaching no significance level (Fig. 2G).

*Analysis of MCPyV DNA*. MCPyV DNA positive cases did not show any statistically significant differences in 3q oncogene *SEC62* expression, probably due to low number of positive

cases (Fig. 2C). There was also no dependency between invasion depth of the tumor in correlation with virus-positive cases, Clark level, necroses, ulceration, relapse or death due to disease (data not shown).

## Discussion

AFX is a mesenchymal neoplasm, rarely seen in daily routine, even in dermatology and dermatopathology units. Relationship of AFX and pleomorphic dermal sarcoma (PDS) is still not clearly identified (43). As AFX is a diagnosis of exclusion, it is mandatory to apply strict diagnostic criteria that include diligent immunohistochemical workup (8-12).

There is growing evidence that viruses play an important role in tumorigenesis, mainly in immunosuppressed patients and an estimated 20% of global cancer burden is related to viral infections (44). Merkel Cell Polyomavirus (MCPyV) was recently detected in Merkel cell carcinoma (MCC) and approximately 91.2% of MCC are MCPyV-positive (41,45). Intriguingly, 85% of all healthy adults and 58% of children younger than 10 years are MCPyV-positive displaying a high seroprevalence of MCPyV (46). In 2010 MCPyV was initially detected within samples of AFX with an incidence of 17% in this study (13). The authors concluded that MCPyV may act as a cofactor in the tumorigenesis of a subset of AFXs (13). In our cohort we were able to detect MCPyV-DNA in 9.7% only. Comparable to Andres *et al*, virus-positive AFXs were detected predominantly in older males with ulcerated tumors (13), but with no correlation to invasion depth or tumor size in our study. At the moment, the role of MCPyV in AFXs remains unclear. Due to high seroprevalence of MCPyV and possibility of viral persistence in several compartments of the body (body fluids, tissue biopsies, several organ specimens) its role in tumorigenesis of AFX remains to be determined (46). MCPyV is frequently detected on healthy human skin (46,47). Therefore, it cannot be excluded that MCPyV detection may only be a bystander phenomenon without clinical implication (46). Intriguingly, in 2016 Liu and colleagues managed to identify human dermal fibroblasts as the primary skin cell type supporting MCPyV gene expression and productive replication after infectious entry (48). Hence, they showed MCPyV infection is promoted via induction of matrix metalloproteinase (MMP) genes by the WNT/β-catenin signaling pathway. It is already known that UV radiation stimulates the WNT signaling and MMP expression (48,49). Taken together molecular pathways with connection to chronic UV exposure and host cell of MCPyV, our findings indicate towards a pathogenetic relationship between infection and AFX. Though, number of MCPyV cases actually is too low to draw clear conclusions.

Prognostic or predictive biomarkers do not exist for AFXs, neither do any histomorphologic parameters exist, that predict clinical outcome and metastatic potential. Of greater importance concerning prognosis than histomorphologic parameters is surgical margin status, as clear margins are associated with improved clinical outcome (50).

In contrast, histomorphologic parameters of prognostic relevance are well defined for malignant melanoma: Histogenetic subtype, Breslow thickness, Clark level, mitotic figures, ulceration, regression and others (51). Results of multivariate analysis of these factors in large melanoma cohorts are reflected in the AJCC (American Joint Committee on Cancer) for melanoma staging and classification from 2009 (52). Hence, in this study we were not able to identify clinical, viral or histomorphological parameters (displaying worse prognosis for instance in malignant melanoma) that correlated with clinical outcome of the patients. Discussion concerning differentiation of AFX from pleomorphic sarcoma is still ongoing.

For the 3q encoded *SEC62* gene, an overexpression was found in a variety of human cancers (21,25,31,32,36,37,39) and in non-small cell lung cancer, cervical cancer as well as head and neck cancer, high *SEC62* expression correlated with a significantly shorter overall survival. These findings indicate a general role of *SEC62* as an oncogene in the pathogenesis of human cancer and emphasize the role of *SEC62* expression level as a prognostic factor in various cancer entities (53). In our study, we found markedly increased *SEC62* expression levels in the lesional tissue compared with the adjacent healthy squamous epithelium in the vast majority of cases pointing towards an oncogenic function of *SEC62* in AFX, as well. While significantly higher *SEC62* expression levels were found in AFX samples that showed intratumoral necrotic areas, which is known to be an adverse prognostic factor in this entity (1), we found no significant correlation of *SEC62* expression with other clinical and histopathological features including the patients' survival. However, given the comparably low number of cases and the only marginal portion of cases of death, these data do not exclude a potential prognostic relevance of Sec62 in AFX which is further indicated by the association of high *SEC62* expression levels with advanced Clark levels and tumor size (see Fig. 2A and G). As it is recommended that tumors displaying prognostically unfavorable features (extension to subcutaneous fat, corresponding to Clark level V, perineural invasion or necrosis), should be diagnosed as PDS. Hence, high expression levels of *SEC62* could serve as a diagnostic parameter for distinction between AFX and PDS. This has to be further investigated with higher number of cases.

Regarding the functional role of Sec62 in tumor cell biology, it was shown that high Sec62 levels can stimulate the migration of tumor cells (21,31,39) as well as their resistance to ER stress (37). If comparable effects can be seen when *SEC62* is overexpressed in AFX cells is a remaining question, which will be ambitious to answer keeping in mind the extremely rare metastasis rate of AFX (1,54) and the fact that there are no established AFX cell lines available.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

CSLM and ML were major contributors in writing the manuscript, made substantial contributions to the conception and design of the study, and gave final approval of the version to be published. LK and FB performed the immunohistochemical staining. TP and SS performed the virological analyzes. SG conducted the statistical tests. TV and BS critically read the manuscript and were major contributors in the study design. All authors read and approve the final manuscript.

## Ethics approval and consent to participate

Investigations were performed after approval by a local Human Investigations Committee (approval no. 281/10; Ethikkomission der Ärztekammer des Saarlandes). All participants gave their informed consent to participate in the study.

## Patient consent for publication

Informed consent for publication of the data was obtained from all patients.

## Competing interests

The authors declare that they have no competing interests.

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