Spinophilin, a multifunctional intracellular protein, has attracted attention within the last years as a novel putative tumor suppressor protein. Recent studies have shown a reduction of spinophilin expression levels in various types of cancer, such as lung adenocarcinoma, ovarian cancer, and chronic myelogenous leukemia. Low expression of spinophilin was also associated with a higher malignant grade in some of these studies. Our own studies on hepatocellular carcinoma (HCC), head and neck squamous cell carcinoma (HNSCC), colorectal adenocarcinoma (CRC) and breast cancer explored the possible role of spinophilin as a proliferative trigger and potentially prognostic factor. We determined the spinophilin expression through immunohistochemistry, quantitative reverse transcriptase-PCR analysis, and used small interfering RNA (siRNA) or stably expressed shRNAs to disable spinophilin in order to investigate the cellular and molecular effects of reduced spinophilin expression. Statistical methods on appropriate cohorts were used to define the prognostic value and impact on clinical outcome of spinophilin expression levels in patients with HCC, HNSCC, or CRC and breast cancer. Spinophilin seems to play an important role as a crucial protein in cell cycle and proliferation, and thus our findings seem to prove this hypothesis derived from other types of cancer. In the cohorts studied, low expression of spinophilin was identified as an independent prognostic factor that indicates poor clinical outcome. In the past, various key molecules and molecular mechanisms highly impacted clinical practice and specific cancer treatment; therefore, novel factors are needed to improve the moderate survival rates of patients with HCC, HNSCC, and CRC. Taken together, spinophilin is a promising new pathophysiological factor and might be a useful novel biomarker for prognostic purposes.

Furthermore, a reduction of spinophilin was noticed in 37% of lung adenocarcinoma and an absence of spinophilin in 20% of these tumors [3]. Concerning these findings, spinophilin was suggested being a novel tumor suppressor in several types of cancer [1].

In our own studies, we have explored the role and influence of spinophilin expression in patients with hepatocellular carcinoma (HCC) [4], head and neck squamous cell carcinoma (HNSCC) [5] and colorectal adenocarcinoma (CRC) [6]. Regarding HNSCC and HCC, our studies were the first to explore the prognostic impact of spinophilin in patients with HNSCC and HCC. In the next paragraphs, we will briefly describe the findings of our studies and discuss them in the context of already published literature on this topic.

Hepatocellular carcinoma (HCC) is one of the most frequent abdominal tumors and among the main leading causes of cancer-related deaths, worldwide [7]. Our study was the first to explore the presence, distribution, and clinical significance of spinophilin expression in HCC [4]. We used immunohistochemistry and quantitative reverse transcriptase-PCR (RT-PCR) to determine the expression of spinophilin. Moreover, we analyzed the correlation of spinophilin expression level and various clinical and pathological characteristics, including the disease-free and cancer-specific survival in 104 patients with primary HCC, by using Kaplan-Meier curves and multivariate Cox proportional models. We also explored the impacts of decreased spinophilin expression concerning the cell cycle by using small interfering RNA (siRNA), in order to silence spinophilin. The proliferation was assessed by using the Ki-67 proliferation marker in cancer tissue. We found a total loss of spinophilin immunoreactivity in 44 of 104 cases (42.3%) and further 37 cases (35.6%) showed reduced levels of spinophilin in comparison with the surrounding non-neoplastic tissue. Low expression of spinophilin holds true as an independent prognostic variable in multivariate Cox regression analysis, with regard to disease-free (hazard ratio (HR) = 1.8; 95% confidence interval (CI) = 1.04-3.40; p = 0.043) and cancer-specific survival (HR = 2.0; CI = 1.1-3.8; p = 0.025). In addition, a higher Ki-67 proliferation marker index was correlated significantly to reduced spinophilin expression in HCC (p = 0.014). Furthermore, a higher cellular growth rate was observed after using siRNA to reduce spinophilin levels and tumour cells showed and increased cyclin D2 expression (p < 0.05).

In our second study on spinophilin in human cancers, we focused on head and neck squamous cell carcinoma (HNSCC), which is associated with high morbidity and mortality rates, especially in advanced tumor stages [7]. HNSCCs are a heterogeneous group of tumors due to different anatomical regions, but they share the same origin in the squamous mucosa. On account of the described heterogeneity, diverse effective treatment modalities and clinical outcomes are observed in patients with HNSCC [8]. It is therefore essential to have a better insight of the oncogenic pathways in order to develop new therapeutic options and improvements in treatment. We evaluated the role of spinophilin and p53 expression by using immunohistochemistry in 85 patients with non-metastatic HNSCC [5]. Reduced levels of spinophilin were observed in 40 tumors (47%) and 9 cases (10.5%) showed a complete loss of spinophilin. A significantly decreased overall survival rate (HR = 1.96; CI = 1.06-3.61; p = 0.030) was verified for patients with reduced spinophilin expression and nuclear p53 staining. The combination of reduced spinophilin expression and alterations in p53 indicates a poor prognosis in HNSCC patients and our study seems to prove the hypothesis of a possible role of spinophilin as a tumor suppressor, which was also described in other types of cancer [1, 3, 4, 6].

Colorectal carcinoma (CRC) is the most common cancer of the digestive system with high morbidity and mortality rates [7]. Although the survival of patients with CRC has significantly improved within the last years due to introduction of novel targeted agents, metastatic CRC still remains incurable in most cases. This indicates that the research of novel prognostic factors and oncogenic pathways is crucial to improve the clinical outcome of patients with CRC. We evaluated the prognostic impact of spinophilin expression in 162 colorectal adenocarcinoma patients using data of the Cancer Genome Atlas [6]. Moreover, we generated stably expressing spinophilin-directed shRNA CRC cell lines and studied the influence of spinophilin expression on cellular phenotypes and molecular interactions. Low spinophilin expression levels are significantly associated with poor prognosis in CRC patients (p = 0.038), a finding that is quite similar to a previously reported study [9]. We confirmed that a reduction of spinophilin levels in p53 wild-type HCT116 and p53-mutated Caco-2 cells were associated with an increase of cellular proliferation and also anchorage-independent growth (p < 0.05). Concerning the molecular level, we explored the idea that low spinophilin levels increase the expression of the transcription factor E2F-1. Moreover, an increase of tumor spheres, CD 133 positive cells and an increased resistance to 5-fluorouracil was observed (p < 0.05).

Very recently, our group published a study of spinophilin in breast cancer patients. Based on patient-related data of almost 1000 patients of the Cancer Genome Atlas, we found out that reduced spinophilin expression is associated with poor survival and aggressive basal-like phenotype. In
addition, growth promoting effects were observed in cell line models and in vivo metastases were promoted. Several potentially differentially expressed genes were discovered by using microarray technology [9].

Taken together, in our studies we evaluated the novel putative tumor suppressor spinophilin in patients with HCC, HNSCC and CRC [14-6]. What these tumors have in common is that they each necessitate the exploration of novel factors, whether useful as drug targets, prognostic markers, or diagnostics in order to improve the moderate survival rates and the clinical outcome for the patients. Spinophilin has attracted attention as a novel putative tumor marker in several types of cancer [1, 3]. Our findings are in line with previously described results from lung adenocarcinoma, chronic myelogenous leukaemia, and ovarian carcinoma, which also showed the negative prognostic impact of low spinophilin expression in patients [1, 3]. Our study on HCC and spinophilin was the first exploration on expression and distribution of spinophilin in HCC [4]. Our findings clearly showed a negative relationship between reduced spinophilin expression and clinical outcome concerning prognosis. Therefore, our data suggests spinophilin as a potentially useful prognostic marker in patients with HCC. However, preclinical studies are needed for the confirmation of spinophilin as a novel marker for prognosis in patients with hepatocellular carcinoma. Regarding the role of spinophilin in CRC, we verified the previously identified association of low spinophilin expression and prognosis in CRC patients [10]. Moreover, we showed that spinophilin might be also involved in cancer stem cells and that low spinophilin levels increase the expression of the transcription factor E2F-1 [6]. Tremendous efforts have been spent on the research of the molecular oncogenic pathways and mechanisms in progression of CRC. This led to the discovery of some key molecules and pathological mechanisms, including vascular endothelial growth factor receptor (VEGF), epidermal growth factor receptor (EGFR), and KRAS/NRAS mutations. On account of that exploration, it is clear that these molecules highly impacted the clinical practice of CRC treatment [11, 12] but, due to the moderate survival rates of metastatic CRC, exploration of novel factors badly needs to continue, whether as drug targets or prognostic markers. Nevertheless, further research and preclinical trials are warranted for validation before the value in clinical practice can be estimated. In our HNSCC study, we described for the first time a reduced spinophilin expression and the negative relationship with disease-free and overall-survival in a substantial number of HNSCC patients [5]. Furthermore, in the majority of HNSCC cases, we found p53 alterations, which, in combination with reduced spinophilin expression, are regarded as a prediction of poor clinical outcome. Therefore, preclinical molecular studies are needed for validation and better understanding. Spinophilin might be useful as a prognostic marker to stratify patients with HNSCC according to prognosis and treatment. In conclusion, we can point out that our findings, regarding the prognostic value of spinophilin, are in line with previously described cancer types [1-3, 10]. Spinophilin, as a putative tumor suppressor, seems to play an important role in pathological mechanisms and molecular pathways as a crucial protein in the cell cycle and proliferation. Thus, our findings seem to prove this hypothesis that was derived from other types of cancer. The discovery of key molecules and pathological mechanisms highly impacted the clinical practice of cancer treatment [10, 11], such as vascular endothelial growth factor receptor (VEGF), epidermal growth factor receptor (EGFR), and KRAS/NRAS mutations. In order to improve the clinical outcome and survival rates of HCC, HNSCC, and CRC patients, novel factors are needed and spinophilin might be useful, but additional preclinical studies are essential for the validation of spinophilin as a novel prognostic tumor marker.

**Conflicting interests**

The authors have declared that no competing interests exist.

**References**

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