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Impaired melanoma growth in VASP deficient mice

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ABSTRACT

Progression of tumors depends on interactions of cancer cells with the host environment. Expression of the cytoskeleton protein VASP is upregulated in various cancer entities. We analyzed the role of VASP for melanoma growth in murine allograft models. Growth of VASP expressing melanomas was retarded in VASP$^{-/-}$ versus wild-type animals. Over time tumor size was <50% in VASP$^{-/-}$ versus wild-type animals and independent of expression levels of Ena/VASP protein family members. Histological analyses showed smaller cells with impaired nutrition status and less vascularization in melanomas derived from VASP$^{-/-}$ versus counterparts from wild-type mice. Cumulatively, the data reveal a critical role of VASP in non-tumor cells in the tumor environment for melanoma growth in vivo.

1. Introduction

Regulation of the actin cytoskeleton plays a central role in cell migration, proliferation, and progression. Directed endothelial cytoskeleton turnover is the driving force for angiogenesis that leads to vascular lumen formation. Steps of elongation and recruitment of endothelial cells to the sites of neovascularization in solid tumors require changes in cell polarity and migration, thus indicating a crucial role of actin-regulatory proteins in cancer growth.

The cytoskeleton protein vasodilator-stimulated phosphoprotein (VASP) is the founding member of the Enabled (Ena)/VASP family and activity of the protein is regulated by complex phosphorylation patterns mediated by cyclic nucleotide- and AMP-dependent protein kinases [1,2]. In mammalians, the Ena/VASP family comprises the mammalian Ena homolog (Mena), VASP, and the Ena-VASP-like protein (EVL). The family members share a tripartite domain organization of an N-terminal Ena/VASP homology 1 (EVH1) domain, a central proline-rich region (PRR), and a C-terminal Ena/VASP homology 2 (EVH2) domain [3]. The EVH2 domain mediates VASP binding to globular actin (G-actin) and filamentous actin (F-actin) [4]. VASP and its phosphorylated forms couple cyclic nucleotide- and AMP-dependent signal transduction pathways to the cytoskeleton and the cytoskeleton-membrane interface [5]. VASP is ubiquitously found in adult and embryonic tissues [5,6], and highly expressed in vascular endothelial cells [7]. In endothelial cells, VASP is localized to actin filaments and enriched in highly dynamic membrane regions and is a crucial factor in the formation and bundling of actin filaments [3]. Overexpression of VASP in the endothelium has been shown to induce stress fiber formation [7], whereas loss of VASP function results in loose cell-cell contacts [2]. Ablation of the VASP gene in mice results in minor if any phenotype [5], whereas combined deficiency in all Ena/VASP proteins is embryo-lethal and mice die during development from edema and bleedings due to defective vessel integrity [8].

Several studies reported that VASP expression levels have been associated with tumorigenesis in cell culture and mouse models

Abbreviations: VASP, vasodilator-stimulated phosphoprotein; Ena, Enabled; Mena, mammalian Ena; Evl, Ena-VASP-like; PRR, proline-rich region; EVH1, Ena/VASP homology 1; EVH2, Ena/VASP homology 2; G-actin, globular actin; F-actin, filamentous actin; WT, wild-type; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; NO, nitric oxide

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[9,10] and VASP expression is up-regulated in human lung carcinoma and increases with more advanced tumor stages. Consistently, elevated VASP expression levels increase invasive migration of human breast cancer cells [10]. As VASP expression is up-regulated in solid malignant cancers, we reasoned that targeting VASP activity might interfere with tumor growth. Here, we challenged VASP$^{-/-}$ and wild-type (WT) mice using the B16 allograft melanoma model. We found that tumor growth was largely impaired in VASP deficient animals. Melanomas in VASP$^{-/-}$ mice contain >3-times more vessels as compared to cancers isolated from WT animals. We conclude that VASP expression in the tumor environment has a critical function for melanoma growth in vivo and that VASP is a potential drug target to interfere with malignant diseases.

2. Materials and methods

2.1. Cell culture

B16 murine melanoma cells were cultivated in DMEM medium supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin (Gibco, Breda, The Netherlands) at 37 °C in a 5% CO_2 atmosphere.

2.2. Mouse melanoma model

VASP$^{-/-}$ mice used in the study were described previously [5]. Control female WT C57Bl/6 mice, 6–8 weeks of age, were purchased from Charles River Wiga (Sulzfeld, Germany). All procedures were approved by local authorities. For tumor induction, 2.5×10^6 B16.F10 melanoma cells were subcutaneously inoculated into the right dorsal flank of VASP$^{-/-}$ and WT mice. All mice in the study were challenged by a single melanoma only. Animals were divided into four groups, which were analyzed at day 8 (7 WT, 2 VASP$^{-/-}$ mice), day 10 (7 WT, 6 VASP$^{-/-}$), day 12 (5 WT, 3 VASP$^{-/-}$), and day 14 (7 WT, 5 VASP$^{-/-}$) after injection. Animals were sacrificed, dark tumor masses were carefully and completely removed en bloc from the surrounding tissue, and weights and sizes of the excised melanoma were determined. Part of the tumor tissue was fixed in phosphate buffered 4% formaldehyde for histological analysis.

2.3. Immunohistological studies

Histological analysis and staining of melanoma in WT and VASP$^{-/-}$ mice were performed as described [13]. Images were taken using a Nikon Coolpix 5000 camera mounted on an Olympus BX51 microscope with a PlanApo 40× objective. To determine the mitotic count ten high power fields (HPF, 400× magnification) of each tumor were evaluated. Tumor vascularization was quantified in analogy to the mitotic count and blood vessels in ten representative HPF were counted.

2.4. Statistical analysis

Results are expressed as mean ± standard deviation (S.D.). Differences between groups were determined by Student's *t*-test. Means and S.D. of melanoma weights were calculated from tumors of 7 WT and 2 VASP$^{-/-}$ mice at day 8; 7 WT and 6 VASP$^{-/-}$ at day 10; 5 WT and 3 VASP$^{-/-}$ at day 12 and 7 WT, 5 VASP$^{-/-}$ at day 14. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. VASP deficiency interferes with melanoma growth

To analyze VASP function for melanoma growth, we used the B16 melanoma model in mice. To trigger allograft melanoma formation, B16 cells were subcutaneously injected into the right dorsal flank of 6–8 weeks old VASP$^{-/-}$ and WT mice, respectively. Solid tumors became palpable around day 6 after cell injection. At day 8, 10, 12, and 14 after B16 cell injection, mice were sacrificed and tumors were carefully resected en bloc from the surrounding tissues, and size and masses of the excised melanoma were determined. At all time-points size (Fig. 1) and weight (Fig. 2) of melanomas were largely reduced in VASP$^{-/-}$mice as compared to WT animals (0.011 ± 0.013 vs. 0.046 ± 0.021 g at day 8 after injection; 0.115 ± 0.056 vs. 0.370 ± 0.111 g at day 10; 0.469 ± 0.290 vs. 0.699 ± 0.138 g at day 12 and 0.864 ± 0.526 vs. 1.666 ± 0.222 g at day 14). Tumor weight in VASP$^{-/-}$ mice was reduced by 23.9%, 31.1%, 67.1%, and 51.9% at day 8, 10, 12, and 14, respectively, as compared to WT mice (Fig. 2).

3.2. Constant Ena/VASP protein expression in growing melanoma

We analyzed endogenous VASP expression in resected melanoma tissue (Fig. 3).

Western blotting using VASP specific antibody M4 showed constant VASP levels in tissue lysates over time. VASP content was similar in tumors growing in VASP$^{-/-}$ and WT mice. Expression of Mena and EVL in the melanoma tissue as assessed by Western blotting or RT-PCR did not change either over time and was indistinguishable in VASP$^{-/-}$ and WT mice (not depicted). The data suggest that growth reduction in melanomas in VASP$^{-/-}$ animals is due to deficiency of exogenous VASP in the tumor environment of the host tissue rather than caused by alterations of endogenous protein in the malignant cells.

3.3. Reduced nutritional status caused by defective angiogenesis in melanoma growing in VASP null mice

To analyze consequences of VASP deficiency in the melanoma-surrounding tissue for tumor growth, we performed histological analysis of formalin fixed and paraffin embedded cross-sections

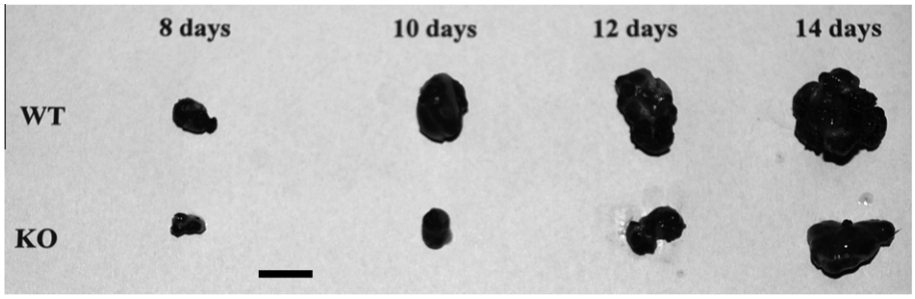

Fig. 1. Melanoma size in WT and VASP$^{-/-}$ mice. Melanoma were resected en bloc from WT and VASP$^{-/-}$ (KO) mice at day 8, 10, 12, and 14 after injection, respectively. The bar corresponds to 1 cm.