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Role of caveolin-2 in subcutaneous tumor growth and angiogenesis associated with syngeneic mouse Lewis lung carcinoma and B16 melanoma models

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Abstract

In addition to cancer cells, primary tumors are composed of a multitude of stromal cell types. Among others, the stromal cell types involved in tumor growth and progression include endothelial cells, fibroblasts, pericytes, stem cells and various cell types of immune origin. While the role of oncogenes or tumor suppressor proteins expressed in cancer cells has been extensively studied, far less is known about potential involvement of proteins expressed in stromal cell types present within the tumor microenvironment. Recent experimental evidence from our laboratory suggests that caveolin-2 (Cav-2) protein expressed in stromal cell types of the tumor microenvironment promotes subcutaneous tumor growth in two independent syngeneic mouse models, i.e., Lewis lung carcinoma (LLC) and B16-F10 melanoma. Mechanistically, the tumor growth promoting role of Cav-2 is associated with enhanced tumor induced neovascularization. At the molecular level, host-expressed Cav-2 appears to prevent excessive expression of anti-angiogenic thrombospondin-1 (TSP-1) and promote phosphorylation of pro-angiogenic endothelial nitric oxide synthase (eNOS) at serine 1177. Taken together, our recent findings suggest that Cav-2 expressed within the tumor microenvironment could be a potential target for anti-cancer therapy.

Keywords

Caveolin-2 (Cav-2); cancer; tumor growth; tumor angiogenesis; Lewis lung carcinoma (LLC); B16 melanoma; thrombospondin-1 (TSP-1); endothelial nitric oxide synthase (eNOS)

It has been well established that tumor growth beyond ca. 2 mm³ requires new blood vessel formation from pre-existing vasculature (angiogenesis) necessary for sufficient tumor tissue oxygenation nourishment [1, 2]. This change from the avascular to the angiogenic phase of tumor growth is frequently referred to as the “angiogenic switch” [1, 2]. Despite numerous studies centered on tumor growth and tumor induced angiogenesis [3-5], the underlining cellular and molecular mechanisms are poorly understood. Caveolins are crucial components

Conflict of Interest

The authors declare that there is no conflict of interest.

of detergent resistant cholesterol lipid rich micro domains including lipid rafts and caveolae. Caveolin-1 (Cav-1) and -2 are ubiquitously expressed and interact with each other, while Cav-3 is muscle specific [6]. Despite similar name, the amino acid sequence between Cav-1 and Cav-2 is 62% different from Cav-1 [7], suggesting distinct functional roles for each of these proteins [8]. A relatively widespread access to Cav-1 knockout (Cav-1 KO) mice independently developed by a number of research laboratories permitted for thorough examination of the role for Cav-1 in tumor growth and tumor-induced angiogenesis [9-14] (Reviewed in [15]). In contrast to Cav-1, the role of Cav-2 expressed within the tumor microenvironment in tumor growth and tumor-induced angiogenesis remained unknown.

In our recent paper using newly developed Cav-2 KO mice we have examined the role of host-expressed Cav-2 in regulating tumor growth and tumor growth-induced angiogenesis using subcutaneously implanted Lewis lung carcinoma (LLC) and B16-F10 melanoma cells as the two independent syngeneic mouse models [16]. Our results showed that LLC tumors implanted into Cav-2 KO mice displayed a defective growth and ultimately regressed, which was in a striking contrast to wild type (WT) mice in which LLC tumors displayed a continuous growth. The robust decline in the volume of Cav-2 KO LLC tumors measured using a caliper in live mice was independently confirmed upon surgical removal followed by weighing and determining the average tumor mass. Moreover, when Cav-2 KO mice implanted with LLC tumors were left for additional 60 days, they still remained tumor-free, suggesting that host-expressed Cav-2 is essential for subcutaneous growth of LLC tumors. Interestingly, in contrast to LLC, subcutaneously implanted B16-F10 melanoma tumors were able to grow in Cav-2 KO mice, however, at significantly reduced rate, suggesting that the degree to which Cav-2 expressed in tumor microenvironment promotes tumor growth may depend on the tumor type.

Since angiogenesis is essential for tumor growth, we postulated that the defective LLC tumor growth in Cav-2 KO mice should be directly linked to reduced microvascular density within tumor tissue. Indeed, immunohistochemical staining with anti-CD31 antibody revealed robustly ca. 13-fold reduced microvascular density within Cav-2 KO LLC tumors on day 10 after implantation. Consistent with less obvious inhibition of growth of B16-F10 tumors in Cav-2 KO, the density of CD31 positive vessels was reduced by only ca. 2.5-fold within B16-F10 tumors implanted into Cav-2 KO mice. Because in addition to severely diminished microvascular density, significantly reduced cell survival and proliferation was observed within Cav-2 KO LLC tumors on day 10 after implantation, we performed subsequent analysis involving the earliest palpable LLC tumors extracted 6 days after implantation. The results of these studies revealed significantly reduced microvascular density within the earliest palpable LLC tumors implanted into Cav-2 KO tumors. However, in contrast to reduced microvascular density, there was no significant reduction in proliferation or cell survival within the earliest palpable LLC tumors extracted 6 days after implantation into Cav-2 KO mice. Thus, our data with diminished microvascular density and yet comparable cell proliferation and survival in the earliest palpable LLC tumors derived 6 days after implantation in Cav-2 KO mice suggest that lack of Cav-2 within stromal cell types of tumor microenvironment results in impaired angiogenesis prior to diminished LLC tumor cell proliferation/survival. Therefore, compromised angiogenesis is the underlying

mechanism responsible for diminished cell proliferation and survival and ultimately defective growth of LLC tumors in Cav-2 KO mice.

Interestingly, despite the robust inhibition of LLC tumor growth and angiogenesis in Cav-2 KO mice, no statistically significant differences in mRNA levels for pro-angiogenic growth factors such as VEGF-A, FGF-2 or PlGF as well as transcriptional targets of Notch pathway Hes-1 and Hey-1 were detected by quantitative real time PCR in Cav-2 KO LLC tumors. However, there was time-dependent increase in mRNA levels of anti-angiogenic thrombospondin-1 (TSP-1) within LLC tumors implanted into Cav-2 KO mice, which reached statistical significance as early as day 6 after LLC implantation. Consistent with significantly higher mRNA levels, the time-dependent increase in TSP-1 protein was determined within LLC tumors implanted into Cav-2 KO mice by immunoblotting. In addition to elevated expression of TSP-1, we also observed robust time-dependent decrease in the levels of phospho-S1177-eNOS within LLC tumors implanted into Cav-2 KO mice. Overall, our data indicate that deficiency of Cav-2 in non-cancer cell types present within LLC tumor microenvironment leads to elevated expression of TSP-1 and inhibition of phospho-S1177-eNOS. Because TSP-1 is one of the key anti-angiogenic molecules which was particularly well characterized in the tumor microenvironment^[17], it is reasonable to suggest that increased levels of TSP-1 within LLC tumors implanted into Cav-2 KO mice is an important molecular mechanism by which lack of Cav-2 in tumor microenvironment suppresses tumor angiogenesis and growth. Whereas TSP-1 was shown to suppress angiogenesis by several mechanisms, inhibition of pro-angiogenic eNOS seems to be particularly important^[17, 18]. Thus a robust suppression of phospho-S1177-eNOS closely correlating with concurrent increase in TSP-1 levels within LLC tumors implanted into Cav-2 KO mice implies that compromised S1177-eNOS phosphorylation is a consequence of inhibitory action of elevated TSP-1 levels present within the tumor microenvironment of Cav-2 deficient mice. Further studies examining the functional consequence as well as molecular and cell-type-specific mechanisms responsible for increased expression of TSP-1 and reduced phosphorylation of eNOS within LLC tumors implanted in Cav-2 deficient mice are clearly warranted.

The recently reported tumor-promoting role of Cav-2 expressed within tumor microenvironment in LLC and B16 models of subcutaneous tumor growth in mice^[16] is in agreement with a number of clinical reports showing upregulation of Cav-2 during progression of several types of human cancer such as esophageal^[19], urothelial carcinoma of the urinary bladder^[20, 21] or prostate cancer^[22]. Cav-2 was also suggested as a prognostic factor in breast cancer^[23, 24] and stage I lung adenocarcinoma^[25]. Thus in light of our recently published basic research data with tumor promoting role of stromal cell-expressed Cav-2 along with clinical data implying prognostic role of Cav-2 in different human cancers, future comprehensive studies dissecting the role of Cav-2 expressed in various stromal cell types of the tumor microenvironment versus cancer cells will be essential. Although the anti-angiogenic therapy is a vital strategy in cancer treatment, owing to commonly developed resistance, pinpointing additional targets will be necessary^[3]. Thus the fact that Cav-2 expressed in host microenvironment promotes tumor angiogenesis implies that this membrane protein may become an important target for anti-angiogenic therapy and cancer treatment.

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