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# Caveolin-1 in Melanoma Progression

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## 1. Introduction

Cancer is a leading cause of death world wide and mortality due to this group of diseases has doubled in the last 20 years. With an estimated 3 million cases, skin cancer is currently the third most common human malignancy and global incidence is rising at an alarming rate due to environmental changes. Within that category, melanomas represent the least common, but most dangerous form accounting for the majority of skin cancer-related deaths.

In general terms, cancer evolves as the consequence of a multi-factorial process that involves the loss of a cell's ability to respond in an appropriate fashion to cues provided by the microenvironment. The development of such aberrant, autonomous behavior is caused by both genetic mutations and epigenetic mechanisms. Particularly relevant in the context of melanoma are the Ras/Raf/MEK/Erk, PI3K/PTEN and NF-kB signaling pathways. The Wnt/β-catenin patway is also implicated, but it´s role still remains unclear. Depending on whether changes result in a "gain of function" or a "loss of function", the molecules involved are classified as either oncogenes or tumor suppressors, examples important in melanomas being NRas and B-Raf or PTEN, respectively. More recently, a new group of molecular participants has begun to emerge, which, depending on the cellular context, display the ability to either block tumor development or favor progression. Very little is still known about the underlying mechanisms that might explain such "ambiguous" behavior.

In this respect, work from our laboratory has focused on the study of a scaffolding protein called caveolin-1. This protein is implicated in a large number of cellular processes, including caveolae formation and vesicular transport, cholesterol transport and the regulation of signal transduction. With respect to tumor development, initial reports implicated caveolin-1 as a tumor suppressor. For instance, caveolin-1 expression is reduced in several human tumors including lung, mammary, colon, ovarian carcinoma and sarcomas, as wells as osteosarcomas and re-expression of the protein can reverse characteristics associated with the transformed phenotype. However, evidence to the contrary is also available showing that caveolin-1 promotes more aggressive traits in tumor cells, such as metastasis and multidrug resistance. Importantly, in human melanoma patients high levels of caveolin-1 are detected in exosomes found in the plasma and some data available associate caveolin-1 expression with increased metastatic potential in different human melanoma cell lines.

In this chapter, we summarize data available in the literature highlighting the ambiguity of caveolin-1 function in cancer development. Mechanisms that might explain one or the other

type of behavior, as well as the possible relevance of caveolin-1 in the development of melanomas will be discussed. Finally, the potential of this understanding for developing therapies will be mentioned.

## 2. Cancer

Cancer is a leading cause of death worldwide that evolves as the consequence of genetic and epigenetic changes (Ponder 2001). This multifactorial process results in the loss of appropriate communication between cells and their microenviroment. During this transition, aberrant cells acquire specific molecular traits, including unlimited replicative potential, resistance to apoptosis, independence of growth factors and insensitivity to growth-inhibitory signals. Insipient tumors require then the formation *de novo* of blood vessels (angiogenesis) to grow beyond an initially limited size. Ultimately, tumor cells develop the ability to disseminate to distant sites and form new tumors (metastasis), which frequently are the cause of patient death. All these processes are thought to reflect the consequence of an inbalance in the activity of genes referred to as oncogenes and tumor suppressors, whereby mutations in both types of genes (genetic regulation), in addition to changes in the levels of expression (epigenetic regulation), contribute to such abnormal development (Weinberg 1989; Hanahan and Weinberg 2000; Hanahan and Weinberg 2011).
Skin cancer is a common disease and the high incidence has generated global concern. Although many of these cancers are not aggressive, some, like melanoma, are extremely lethal. As indicated, aberrant cellular signaling underlies the development of essentially all tumors. Not surprisingly, therefore, in the transition from melanocytes to melanomas, many signaling pathways become constitutively activated, as will be discussed later and promote the acquisition of molecular traits associated with melanoma development. (Figure 1).
Malignant melanomas are a serious public health problem in many countries; however, data from less developed countries is often either not available or not reliable. The data available in developed countries reveals that mortality associated with melanomas has increased gradually in the past 40 years. Perhaps more problematic in this context is the fact that the incidence rate is increasing faster in younger people, an aspect which tends to amplify the impact of this deadly cancer. Malignant melanoma is known to initially develop in melanocytes located at the interphase between epidermis and dermis. These peripherally located, proliferative cells suffer damage upon excessive UV exposure that can translate ultimately into cell transformation and invasion of deeper structures of the skin. While still at an early stage of development, equivalent to less than 4 mm of vertical penetration and the absence of distant spread, surgical resection represents an appropriate treatment for melanomas. However, in more advanced cases, the success of surgical and therapeutical procedures is severely limited by tumor recurrence, metastasis and relatively rapid patient death. Hence, in order to develop more effective treatments, it is important to understand better the mechanisms leading to melanocyte damage and, particularly, to identify those responsible for the alterations in signaling that initiate and propagate the transformation process. In recent years, a large body of evidence has accumulated identifying sun exposure as a mayor risk factor in developing this disease. However, simply eliminating this risk by protective measures is not an option, since sun exposure is necessary for a number of reasons, including being required for vitamin D synthesis. Hence, it is important to define when precisely sun exposure becomes deleterious, in order to identify groups of the population at risk and thereby improve early diagnosis and treatment.

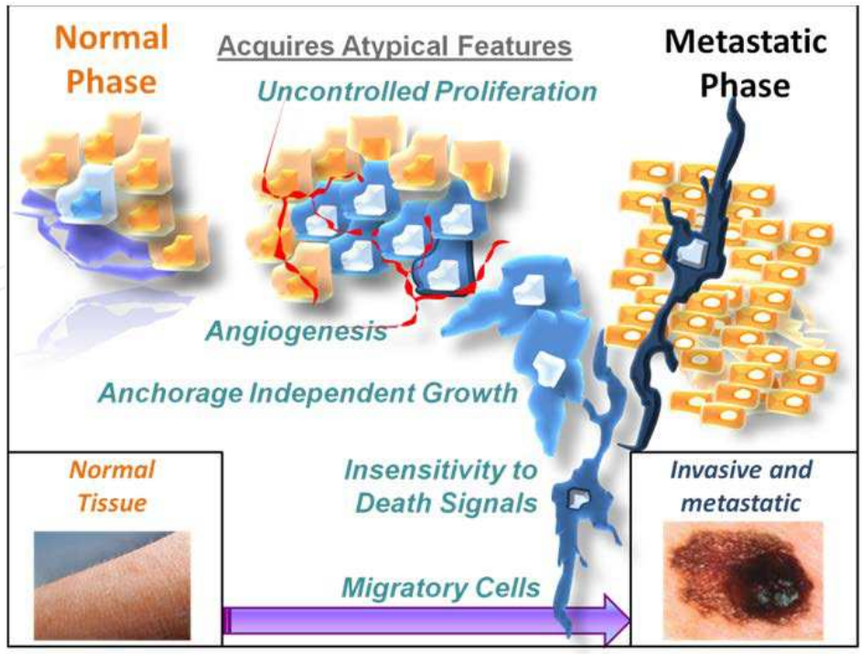



Fig. 1. Atypical characteristics acquired by melanocytes in the transition to malignant melanomas

Melanocytes are intimately associated with keratinocytes in the intermediate cell layer between dermis and epidermis. The acquisition of atypical features, such as uncontrolled proliferation, angiogenesis, anchorage-independent growth and insensitivity to death-inducing signals are depicted. Additionally, the cells eventually begin to migrate, become invasive and metastatic. These cells invade both the dermis and epidermis layers, thereby increasing the size of the primary tumor before disseminating via the blood stream to other tissues.

## 3. Melanoma incidence

The International Agency for Research on cancer publishes a document entitled "Cancer Incidence in Five Continents", which permits comparisons between countries. In general terms, countries with white/Kaukasian-populations have the highest incidence rates (above 2/100.000) in the world, while asians, africans and indigenous americans are much less at risk (1-0.1/100.000). The highest registered incidence for melanomas in the world is found in Australia, whereby a latitude gradient exists, such that rates are highest in the northern part of the country (over 50/100.000 in men, and 40/100.000 for women). Also in New Zealand the incidence of 30/100.000 is well above the global average and higher to the north. Given that in both these countries the predominantly white population is homogeneously distributed, differences is incidence rates can be largely explained by the latitude gradient in sun exposure. Amongst european countries, Scandinavia and Switzerland have the highest incidence rates, being in the latter case 16/100.000 for men and 19/100.000 for women. For Scandinavian countries, there is no straightforward explanation, although the population is extremely fair, because sun exposure is low. In Switzerland, ozone depletion may be a relevant factor. Interestingly, Italy also has latitude a gradient which is inverted compared

to the one in Australia, such that for residents of northern regions (less sun exposure) incidence rates are higher than in southern regions with increased exposure. The question arising here is whether a "skin gradient" exists within the Italian population. Also in the USA incidence rates are quite high, but only in non-hispanic, white populations (19/100.000 men, 16/100.000 women). Relevant factors are again the ethnic background and sun exposure. However, no latitude gradient has been described in the USA. For the rest of the world, the incidence appears low. However, data is frequently either not available or not reliable, in the latter case mainly due to confusion with non-melanoma skin cancer.

A this point, we wish to dwell briefly on the problem of malignant melanoma in Chile. This narrow and extremely long country (approx. 200 by 5000 km) extends from 18°S to 55°S and covers, therefore, geographical zones particularly in the center and to the north with intense sun exposure. Moreover, UV irradiation has also increased dramatically in the southern-most region (ie: XII region) close to the Antarctic, due to ozone depletion that has become evident since the 1970s. Consistent with this development, epidemiological data accumulated over the last 30-40 years indicate a notable increment in the incidence of melanomas also in these areas. The genetic background of the population is fairly homogeneous, being predominantly a mixture between the indigenous population of asian origin and europeans, whereby interracial mixing has occurred over the last 500 years. The population is homogeneously distributed, such that this factor does not contribute to the latitude gradient. In any case, available epidemiological studies do not segregate data according to ethnic background.

Malignant melanoma is a relevant public health problem given the prevalence throughout the country and the relatively high mortality rate associated with this disease (slightly above 1:100.000 deaths/year). Currently, melanomas are the 9$^{th}$ most frequent cause of cancer death in Chile. Unfortunately, as mentioned previously, for a large number of countries in the world, no useful incidence data is available due to registration problems and confusion with non-melanoma skin cancer. The mortality rate within the country ranges from about 3.0/100.000 inhabitants (in the northern-most region of Chile) down to 1.2/100000 in the southern region (XI region). However, in the region furthest south in Chile (XII), death rates are again higher and reach values of approximately 2.1/100.000. For malignant melanoma, certain differences in body distribution are observed for Chile in comparison to melanomas in Europe, North America, Australia and New Zealand. In Chile the most frequent cases are of the acral-lentiginous type, that are observed in non sun-exposed regions of the skin, such as hand palms, foot soles and under the nails. For the other countries mentioned, in most cases the body, face and arms are affected, suggesting sun exposure as an important causing factor. Treatments for malignant melanoma in Chile are similar to those employed in the other countries. Generally, surgical resection of large areas, with or without removal of sentinel lymph nodes, remains the procedure of choice.

## 4. Melanoma biology

Melanoma is a highly complex disease that involves activation of oncogenes, such as BRAF, NRAS, the Wnt pathway and loss or mutation of tumor suppressors, such as p53 or PTEN. For the subsequent discussion, we will focus in this chapter on the possible contribution of the Wnt/β-catenin pathway to melanoma development. Excellent reviews concerning the other alterations mentioned can be found elsewhere (Straume and Akslen 1997; Larue and Delmas 2006; Gray-Schopfer, Wellbrock et al. 2007).

## 5. The Wnt/β-catenin pathway

The canonical Wnt pathway is involved in the control of a large variety of processes, including cell proliferation, cell migration and differentiation, all key events in the initiation and progression of cancer. A crucial component of this pathway is β-catenin (Bienz and Clevers 2000; Polakis 2000; Widelitz 2005) (Klaus and Birchmeier 2008).

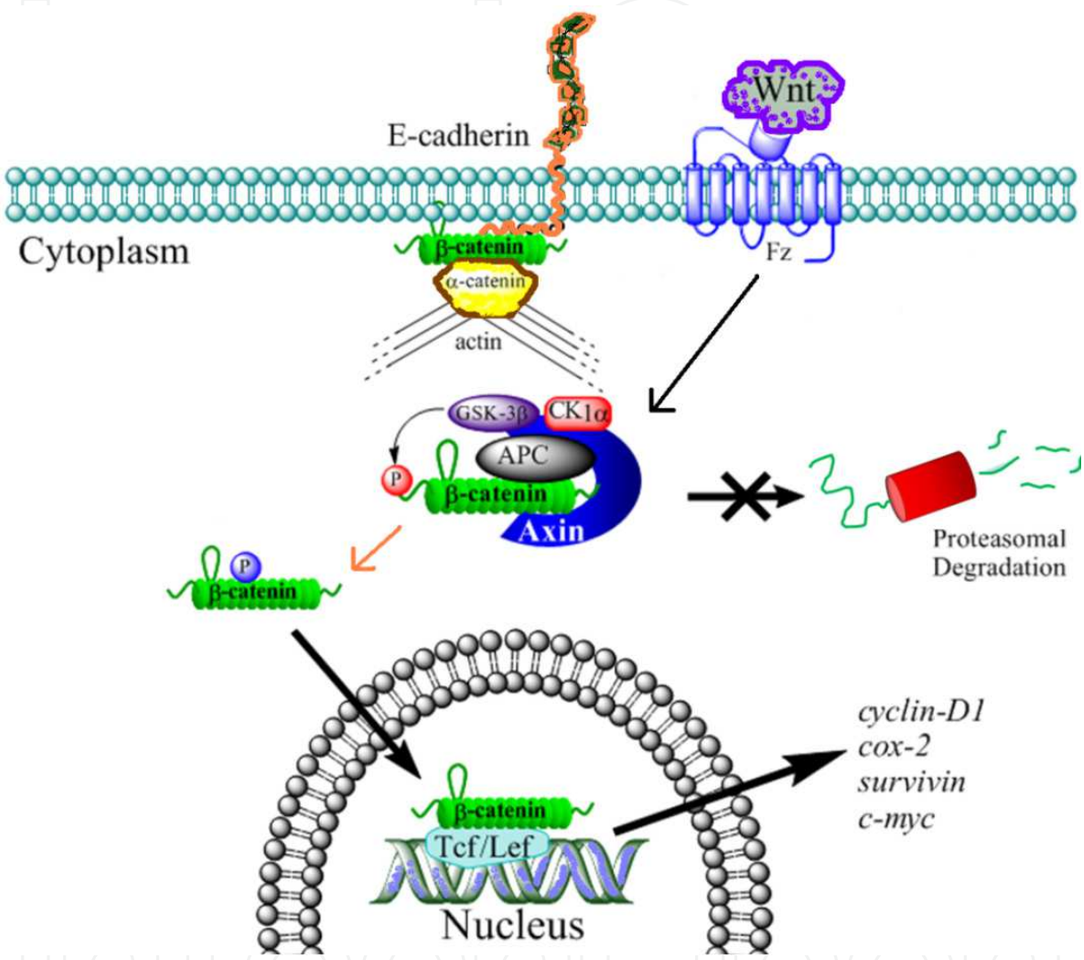



Fig. 2. Wnt/β-catenin pathway.

Schematic showing some of the relevant components of the pathway and highlighting three key locations of β-catenin in the cell. On the one hand, β-catenin is found at the plasma membrane in a multiprotein complex with E-cadherin important for cell-cell interactions. Additionally, β-catenin is present in the cytosol, generally associated with another multi-protein complex that includes adenomatous polyposis coli (APC), axin and GSK-3β and is responsible for promoting degradation via the proteasome pathway. Finally, β-catenin is also present within the nucleus, where it acts as a cofactor that promotes transcription by Tcf/Lef family members of a large number of genes including *survivin, cox-2, cyclin D1 and c-myc*.

In non-stimulated cells, cytoplasmic free β-catenin is associated within a multiprotein complex containing Axin/Conductin, glycogen synthase kinase 3β (GSK3β/Shaggy) and the

tumor suppressor APC (Bienz and Clevers 2000; Polakis 2000; Henderson and Fagotto 2002; Nathke 2004; Heeg-Truesdell and LaBonne 2006). In this complex, Axin/Conductin acts as a scaffolding protein that binds APC, GSK3β and β-catenin, thereby promoting the phosphorylation of APC and β-catenin. Axin also associates with the protein kinase CK1, which phosphorylates β-catenin prior to GSK3β engagement and thereby promotes subsequent GSK3β-dependent phosphorylation, in a process referred to as hierarchical phosphorylation (Heeg-Truesdell and LaBonne 2006). Subsequent phosphorylation of β-catenin by GSK3β drives β-catenin ubiquitination by the SCF complex, thus promoting proteasome-mediated degradation of β-catenin (Kimelman and Xu 2006).

Following Wnt stimulation of Frizzled receptors, Dishevelled is recruited to the receptor. Dishevelled binding serves to help maintain the Axin/APC/GSK3β protein complex in the vicinity of the Frizzled receptor. Additionally, this interaction favors release of β-catenin from the degradative Axin/APC/GSK3β protein complex, hence preventing proteasomal-mediated degradation of β-catenin (Logan and Nusse 2004; Huang and He 2008). Then, β-catenin translocates and accumulates in the nucleus, where it forms a protein complex with T-cell factor/ lymphoid enhancer factor (Tcf/Lef) transcription factors and thereby promotes transcription of Wnt target genes, such as *cyclin D1, survivin, cox-2, c-myc* (He, Sparks et al. 1998; Shtutman, Zhurinsky et al. 1999; Haertel-Wiesmann, Liang et al. 2000; Kim, Plescia et al. 2003).

As indicated (Figure 2), another pool of β-catenin is present in adhesion junctions, where β-catenin forms a protein complex with E-cadherin, α-catenin and actin. This protein complex has been suggested to mediate cell adhesion and confer mechanical stability to cells (Gottardi, Wong et al. 2001; Brembeck, Rosario et al. 2006; Jeanes, Gottardi et al. 2008; Schmalhofer, Brabletz et al. 2009). By sequestering β-catenin within the plasma membrane, E-cadherin reduces β-catenin-Tcf/Lef-dependent transcription in a manner that is independent of its function as a cell-cell adhesion molecule (Gottardi, Wong et al. 2001). Consistent with this view, loss or inactivation of E-cadherin increase responses to Wnt-Ligands (Jeanes, Gottardi et al. 2008). Thus, E-cadherin is a negative regulator of the Wnt pathway and a strong inhibitor of cancer progression (Ma, Young et al. ; Berx, Nollet et al. 1998; Gottardi, Wong et al. 2001; Hajra and Fearon 2002; Margineanu, Cotrutz et al. 2008).

## 6. Alterations in the Wnt/β-catenin pathway in melanoma

The contribution of the Wnt/β-catenin pathway to melanoma development is highly controversial. Some authors suggest that Wnt/β-catenin activation promotes melanoma development (Rimm, Caca et al. 1999; Larue and Delmas 2006; Larue, Luciani et al. 2009). This view is supported by the observation that alterations in the Wnt/β-catenin pathway are detectable in 70-80% of melanomas. Specifically, mutations in β-catenin have been detected in both melanoma biopsies and melanoma cell lines (Larue and Delmas 2006). These findings are reinforced by immuno-histochemical studies showing that β-catenin staining is frequently observed in primary melanomas, but is less abundant in metastatic melanomas (Pecina-Slaus, Zigmund et al. 2007; De Panfilis, Ferrari et al. 2009) and appears to be inversely correlated with the Clark stage (Pecina-Slaus, Zigmund et al. 2007). In both cases, β-catenin is observed predominantly at the cell membrane and in the cytoplasm (Pecina-Slaus, Zigmund et al. 2007; Tucci, Lucarini et al. 2007; De Panfilis, Ferrari et al. 2009). Furthermore, β-catenin has been detected in the nucleus in 30% of the melanomas analyzed (Rimm, Caca et al. 1999). It is important to note, that APC alterations are not frequently

observed in melanomas in comparison to colon cancer (Lucero, Dawson et al. ; Larue and Delmas 2006). However, based on such evidence it remains poorly understood how aberrant β-catenin function may be related to melanoma progression. Possibly, its function in this sense is dependent on direct association of β-catenin with the promoter of Microphthalmia-associated transcription factor (MITF), which is frequently amplified in melanoma cell lines (Garraway, Widlund et al. 2005) and is required for Wnt/β-catenin to induce melanoma growth (Widlund, Horstmann et al. 2002). Interestingly, MITF has been proposed to contribute to melanoma development, by promoting expression of target genes, such as cdk2 that favors cell-cycle progression, anti-apoptotic *bcl2,* and *c-met* that enhances cell motility (Cheli, Ohanna et al. ; Levy, Khaled et al. 2006). Consistent with this view, reduction in MITF levels sensitizes melanoma cells to chemotherapy-induced cell death (Garraway, Widlund et al. 2005).

Alternatively, other groups propose that β-catenin prevents melanoma progression (Lucero, Dawson et al. ; Kageshita, Hamby et al. 2001; Maelandsmo, Holm et al. 2003; Bachmann, Straume et al. 2005; Chien, Moore et al. 2009). This view is supported by clinical data associating more aggressive melanomas and melanoma progression with loss of β-catenin (Kageshita, Hamby et al. 2001). Consistent with this view, loss of cytoplasmic β-catenin correlates with increased thickness of lesions and reduced disease-free patient survival (Maelandsmo, Holm et al. 2003). Additionally, loss of nuclear β-catenin is associated with a reduction in patient life span (Bachmann, Straume et al. 2005). Alternatively, activation of β-catenin is associated with reduced tumor cell proliferation (Chien, Moore et al. 2009). Taken together, these observations favor the notion that β-catenin plays a protective role in melanoma development. To date, however, neither how β-catenin develops such a role nor the mechanisms that lead to loss of β-catenin are fully understood.

A possible explanation for these striking differences in comparison to colon cancer may be related to the type of Wnt ligand present. Analysis of melanocytic tumors reveals that levels of the Wnt ligands 3A and 5A are increased (Larue and Delmas 2006). However, these two Wnt ligands are thought to have opposite functions. *In vitro* assays show that Wnt 3A reduces cell proliferation in association with activation of the β-catenin pathway. Microarray analysis of 350 melanoma samples showed that high levels of β-catenin correlated with better patient prognosis, suggesting that activation of β-catenin pathway by Wnt 3A reduces melanoma cell proliferation (Chien, Moore et al. 2009). How activation of the Wnt/ β-catenin pathway causes such effects in melanoma cells remains unclear, particularly since the opposite is seen in other cell types (Giles, van Es et al. 2003; Logan and Nusse 2004; Khan, Bradstock et al. 2007).

Alternatively, Wnt 5A expression in melanomas correlates with tumor progression and metastasis (McDonald and Silver 2009), and Wnt 5A has been shown to increase melanoma cell migration (Dissanayake, Wade et al. 2007). Interestingly, these effects of Wnt 5A have been described both in *in vitro* and *in vivo* melanoma models (Dissanayake, Olkhanud et al. 2008). In general, Wnt 5A enhances-melanoma cell migration but does so in a manner independent of the canonical Wnt/β-catenin pathway (Dissanayake, Wade et al. 2007; Dissanayake, Olkhanud et al. 2008; O'Connell and Weeraratna 2009).

Another important factor that contributes to changes in the Wnt/β-catenin pathway is the tumor suppressor E-cadherin (Margineanu, Cotrutz et al. 2008; Wu, Lin et al. 2008). Mutations or loss of this cell-cell adhesion protein are associated with increases in β-catenin Tcf/Lef-dependent transcription and cell proliferation (Berx, Nollet et al. 1998). Thus, loss of E-cadherin generally correlates with poor patient prognosis. Moreover, changes in the

expression pattern of Cadherins are relevant. For instance, replacement of E-cadherin by N-cadherin is linked to the epithelial-mesenquimal transition and acquisition of a more malignant cell phenotype (Gray-Schopfer, Wellbrock et al. 2007; Kreizenbeck, Berger et al. 2008)

## 7. Caveolin-1

Caveolins are a family of membrane-associated scaffolding proteins implicated in variety of functions in cells, including vesicle trafficking, cholesterol transport and regulation of signal transduction (Anderson 1998; Okamoto, Schlegel et al. 1998; Quest, Leyton et al. 2004). To date three major isoforms have been described in mammals, namely caveolin-1, -2 and -3 (18-24 kDa). All three isoforms are encoded by distinct genes (Williams and Lisanti 2004). While caveolin-1 and -2 are fairly generically expressed, caveolin-3 presence is limited to muscle and glial cells (Okamoto, Schlegel et al. 1998; Razani, Schlegel et al. 2000; Williams and Lisanti 2004). Different variants have been described for caveolin-1 and -2 (Razani, Woodman et al. 2002; van Deurs, Roepstorff et al. 2003).

Since caveolin-1 is the best characterized isoform, the discussion here will focus on this protein. Two variants referred to as caveolin-1α and-1β have been described that are generated by alternative initiation or splicing (Scherer, Tang et al. 1995; Kogo and Fujimoto 2000; Kogo, Aiba et al. 2004). Caveolin-1β lacks the first 31 amino acids present in caveolin-1α. This region contains the amino acid tyrosine 14, which is phosphorylated by src family kinases (Cao, Courchesne et al. 2002; Labrecque, Nyalendo et al. 2004). Caveolin-1 assumes an unusual topology, whereby a central hydrophobic domain (residues 102-134) is thought to form a hairpin structure within the membrane (Figure 3). As a consequence, both the N-terminal (residues 1-101) and C-terminal domain (residues 135-178) face the cytoplasm. A 41-amino acid region in the N-terminal domain, as well as COOH-terminal elements are required for the formation of caveolin-1 homo-oligomers. In the C-terminal segment, three palmitoylation sites are present at positions 133, 143 and 156 (Figure 3).

Caveolin-1 is phosphorylated on tyrosine-14 in response to growth factors like insulin (Mastick, Brady et al. 1995; Mastick and Saltiel 1997; Lee, Volonte et al. 2000; Kimura, Mora et al. 2002) or EGF (Lee, Volonte et al. 2000; Orlichenko, Huang et al. 2006) and by extracellular stimuli including, UV, oxidative stress or hyperosmolarity (Li, Seitz et al. 1996; Volonte, Galbiati et al. 2001; Sanguinetti and Mastick 2003; Cao, Sanguinetti et al. 2004). These observations have implicated caveolin-1 and particularly phosphorylation on tyrosine 14 in cellular stress responses. Consistent with this notion, caveolin-1 knockout mice have a reduced lifespan and are less resistant to partial hepatectomy (Park, Cohen et al. 2003; Fernandez, Albor et al. 2006).

Caveolin-1 and phosphorylated caveolin-1 are also implicated in cell migration. A specific aminoacid sequence (aminoacids 46-55) is required for localization of the protein to the rear of migrating cells (Sun, Flynn et al. 2007; Sun, Liu et al. 2009) and such polarized distribution of caveolin-1 and associated cell signaling elements is considered important for directional migration of some cell types (Isshiki, Ando et al. 2002; Parat, Anand-Apte et al. 2003; Beardsley, Fang et al. 2005). Although phosphorylation of caveolin-1 on tyrosine 14 has been shown to favor migration via a process involving recruitment of the adaptor protein Grb7 (Lee, Volonte et al. 2000), the precise role of caveolin-1 in these events remains an issue of controversy. In part this is attributable to technical problems associated with the precise identification of phospho-caveolin-1 localization in migrating cells (Hill, Scherbakov et al. 2007).

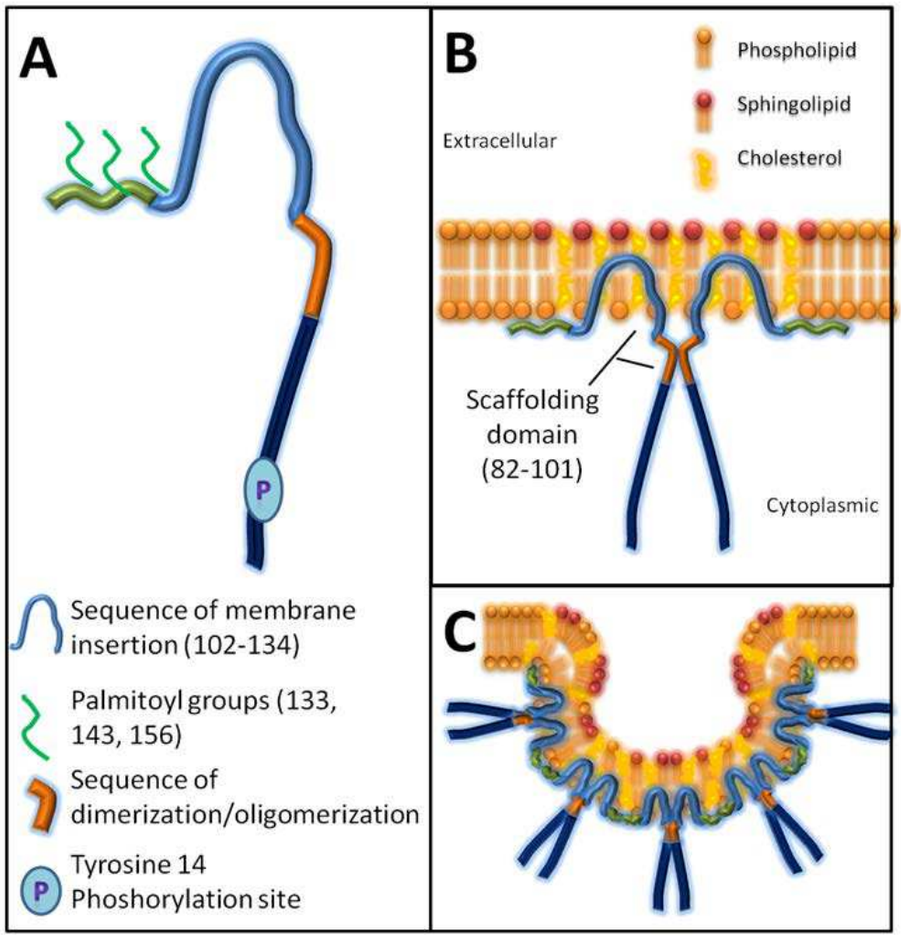



Fig. 3. Caveolin-1 structure and caveolae morphology.

A) Schematic showing the different domains present in caveolin-1 that permit interaction with other proteins and membranes. B) Caveolin-1 anchorage via the membrane insertion domain into regions enriched in sphingolipids and cholesterol. C) Caveolin-1 oligomerizes to generate the proteinaceous coat of caveolae, small invaginations (50-100 nm) of the plasma membrane. Additional proteins called cavins are also essential for the formation of these structures (not shown).

Despite these issues, a large body of literature is available linking the expression of caveolin-1 not only to enhanced migration but also metastasis of cancer cells. Likewise, caveolin-1 is implicated in development of the multi-drug resistant phenotype of aggressive cancer cells. All three characteristics of caveolin-1 mentioned, namely its participation in cellular stress responses and regeneration, migration and metastasis, as well as multi-drug resistance tend to favor the interpretation that caveolin-1 represents a protein whose presence is associated with tumor progression. Such evidence, however, has generated an intense discussion concerning the precise role of caveolin-1 in cancer, since also a large body of data is available suggesting that caveolin-1 functions as a tumor suppressor (see subsequent sections). A key objective in the remaining section of this chapter will be to highlight important aspects of this ongoing discussion and attempt to reconcile these different and opposing functions of caveolin-1 in a working model (see Figure 4). In doing so, we will

focus our attention mostly on studies dealing with the role of caveolin-1 in modulating the Wnt/β-catenin pathway.

## 8. Role of caveolin-1 as tumor suppressor: Inhibition of β-catenin-Tcf/Lef-dependent gene expression

Over the last 15 years, a large amount of data has become available associating the presence of caveolin-1 with tumor suppression. However, as will be discussed, the ability of caveolin-1 to act in this fashion depends on the cellular context. Thus, caveolin-1 should be considered a "conditional" tumor suppressor. In initial studies, oncogene-mediated transformation of NIH3T3 fibroblasts was shown to correlate with reduced caveolin-1 mRNA and protein levels, and re-expression of the protein was sufficient to revert cell transformation (Koleske, Baltimore et al. 1995; Engelman, Wykoff et al. 1997). Likewise selective loss of caveolin-1 expression using an siRNA approach was sufficient to transform NIH3T3 fibroblasts (Galbiati, Volonte et al. 1998). Furthermore, caveolin-1 expression is reduced in a number of human tumors, including lung, mammary, colon and ovarian carcinomas, as well as osteosarcomas (Lee, Reimer et al. 1998; Racine, Belanger et al. 1999; Bender, Reymond et al. 2000; Wiechen, Diatchenko et al. 2001; Wiechen, Sers et al. 2001; Bender, Montoya et al. 2002; Ho, Huang et al. 2002; Cantiani, Manara et al. 2007). Here too, re-expression of caveolin-1 frequently, but not always, reverts characteristics associated with the transformed phenotype (Yang, Truong et al. 1998; Li, Yang et al. 2001; Tahir, Yang et al. 2001; Karam, Lotan et al. 2007; Yang, Addai et al. 2007; Bartz, Zhou et al. 2008). More recently, decreased caveolin-1 levels have been reported for lymph node metastases from head and neck squamous cell carcinoma and restoration of caveolin-1 expression suppresses growth and metastasis (Zhang, Su et al. 2008).

Despite the fact that caveolin-1 depletion in knockout mice does not lead to drastic changes in viability, it is now clear that caveolin-1 absence favors lung and mammary hyperplasia, angiogenesis, as well as carcinogen induced tumor formation in skin tissue (Drab, Verkade et al. 2001; Razani, Engelman et al. 2001; Capozza, Williams et al. 2003; Williams, Cheung et al. 2003). Also, increased mammary and intestinal stem cell proliferation is observed in caveolin-1 knockout mice and caveolin-1 was also shown to control neural stem cell proliferation (Jasmin, Yang et al. 2009). Finally, stromal expression of caveolin-1 in breast cancer predicts outcome, recurrence and survival, further highlighting its relevance as a potential therapeutic target (Sloan, Ciocca et al. 2009; Witkiewicz, Dasgupta et al. 2009). Indeed, caveolin-1 mutation of P132L, which was previously linked to breast cancer (Hayashi, Matsuda et al. 2001), was recently demonstrated to predict recurrence and metastasis in an orthotopic mouse model (Bonuccelli, Casimiro et al. 2009). Taken together, these reports demonstrate that caveolin-1 displays traits consistent with a role of the protein as a tumor suppressor. This ability of caveolin-1 has often been linked to inhibition of signalling events associated with cell survival and proliferation. However, it is important to note that alternative mechanisms have also been proposed. For a more detailed discussion of literature related to the tumor suppressor hypothesis, the interested reader is referred to additional reviews (Williams and Lisanti 2005; Quest, Gutierrez-Pajares et al. 2008).

Initially, our entrance to the caveolin-1 field came with the demonstration that caveolin-1 protein levels are reduced both in tumors from patients with colon cancer, as well as in colon adenocarcinoma cells and that caveolin-1 functions as a tumor suppressor *in vivo* upon re-expression in different colon adenocarcinoma cells (Bender, Reymond et al. 2000; Bender,

Montoya et al. 2002). Despite the ever-increasing abundance of signaling molecules available in the literature for regulation by caveolin-1, at the time relatively few were linked to specific transcriptional events. Thus, as one approach, we set out to compare, by microarray analysis, colon cancer cell lines expressing or not caveolin-1. Rather intriguingly, those studies identified in an initial screen the IAP protein survivin as one of the most strongly down-regulated targets at the transcriptional level (Torres, Tapia et al. 2006). This protein is of tremendous interest, since it is abundantly expressed in a variety of human tumors including lung, colon, breast, prostate, pancreatic, and gastric carcinoma, but is essentially absent in most normal tissues. Importantly, survivin expression in cancer cells is linked to tumor survival. These characteristics define survivin as a tumor specific antigen (Li, Ambrosini et al. 1998; Reed 2001; Altieri 2003).

The mayor challenge then was identifying a mechanism that permitted connecting events thought to occur at the plasma membrane with transcription in the nucleus. Given the importance of canonical Wnt signaling in colon cancer, this pathway became an attractive potential caveolin-1 target. This possibility was further substantiated by reports showing on the one hand that caveolin-1 expression prevented transcription of cyclinD1 by sequestering β-catenin (Hulit, Bash et al. 2000) and on the other that survivin is a β-catenin/Tcf/lef target gene (Kolligs, Bommer et al. 2002). With this in mind, we then established that caveolin-1 controled survivin expression by sequestering β-catenin to the plasma membrane (Torres, Tapia et al. 2006) and subsequently that this ability required the expression of E-cadherin in both colon cancer and melanoma cell lines (Torres, Tapia et al. 2007). Finally, evidence was obtained showing that caveolin-1 regulates in a similar fashion also the expression of cyclooxygenase-2 (COX2; (Rodriguez, Tapia et al. 2009).

Since loss of E-cadherin is frequently observed in human epithelial tumors (Cavallaro and Christofori 2004), our studies suggest that the combined loss of caveolin-1 and E-cadherin in epithelial cells is likely to promote increased expression of genes relevant to epithelial-mesenchymal transition, loss of cell-cell contacts and cell transformation. Perhaps even more importantly, they provide mechanistic insight to how caveolin-1-specific suppression of genes associated with its role as a tumor suppressor becomes "conditional", that is dependent on the cellular context (Torres, Tapia et al. 2007; Quest, Gutierrez-Pajares et al. 2008). Interestingly, this ability of caveolin-1 is not only limited by the proteins present within cells expressing caveolin-1, but also by factors present in the cellular medium. In particular, caveolin-1 expression was shown to limit $PGE_2$ accumulation in the culture media of HEK293T, DLD-1 and HT29 cells. Alternatively, supplementation of media with $PGE_2$ disrupted caveolin-1 complexes responsible for sequestration of β-catenin to the plasma membrane (Rodriguez, Tapia et al. 2009).

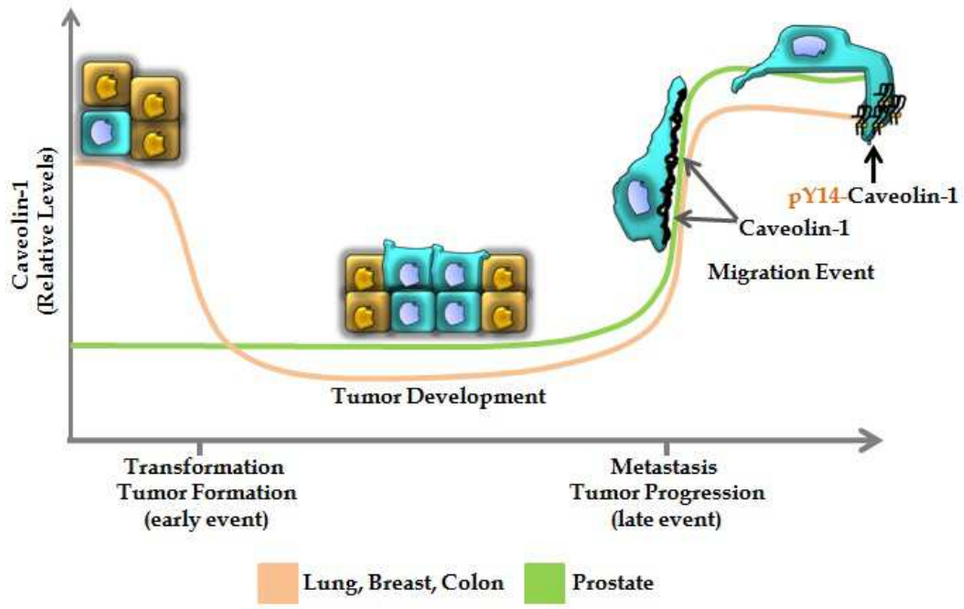
Despite the abundance of evidence summarized previously indicating that caveolin-1 functions as a tumor suppressor, there is also evidence suggesting a radically different, even opposite role for caveolin-1. Specifically, caveolin-1 is known to promote tumor formation and presence is correlated with poor prognosis and survival in prostate cancer. Indeed, the expression of caveolin-1 reportedly increases in primary tumors from prostate (Yang, Truong et al. 1998) and certain leukemia derived cell lines (Hatanaka, Maeda et al. 1998). Also, in prostate cancer cells caveolin-1 presence increases tumor growth and the incidence of metastasis (Li, Yang et al. 2001; Tahir, Yang et al. 2001; Karam, Lotan et al. 2007; Bartz, Zhou et al. 2008). Increased caveolin-1 expression in tumor samples is not restricted to cases, like the prostate, where normal tissues have low relative caveolin-1 levels, since increased expression was also reported in tumor models where initial caveolin-1 loss is observed, such

as colon (Bender, Reymond et al. 2000) and breast cancer (Fiucci, Ravid et al. 2002; Garcia, Dales et al. 2007; Savage, Lambros et al. 2007).
In most of these cases, the available data argue for a strong positive correlation between expression of caveolin-1, metastasis and multidrug resistance (Lavie, Fiucci et al. 1998; Lavie and Liscovitch 2000; Garcia, Dales et al. 2007). Moreover, studies in samples derived from esophageal squamous cell carcinoma (Ando, Ishiguro et al. 2007), small cell lung carcinomas (Ho, Huang et al. 2002), colon cancer cells with elevated metastatic potential ((Bender, Reymond et al. 2000), see below) and gastric cancer (Burgermeister, Tencer et al. 2003), revealed that caveolin-1 expression correlates with poor patient prognosis. Furthermore, caveolin-1 is also overexpressed in nasopharyngeal carcinoma and protein levels correlate there with poor prognosis, enhanced tumor cell migration and metastasis (Du, Hu et al. 2009). Finally, caveolin-1 was recently associated with tumor progression in a panel of melanoma cell lines, since increased expression correlated with enhanced proliferation, cell migration and tumorigenicity (Felicetti, Parolini et al. 2009).

## 9. Caveolin-1 function as a promoter of metastasis: The role of phosphorylation on tyrosine-14

A variety of potential mechanisms have been invoked to explain how caveolin-1 presence may favor tumor progression. For example, in prostate cancer cells, increased caveolin-1 levels were found to favor growth factor release and regulation by a positive feedback loop that enhances tumor cell invasiveness (Li, Ren et al. 2009) and VEGF-associated pro-angiogenic signaling (Tahir, Park et al. 2009). Futhermore, Caveolin-1 is located on prostasomes secreted by prostate cancer cells (Llorente, de Marco et al. 2004) and the presence of antibodies against caveolin-1 in blood plasma decreased prostate cancer cell metastasis in animal models (Watanabe, Yang et al. 2009). In breast cancer cells, caveolin-1 was recently shown to associate with type 1 matrix metalloproteinase and thereby promote invadopodia formation, as well as matrix degradation, both of which favor invasiveness (Yamaguchi, Takeo et al. 2009). Also, caveolin-1 enhanced hepatocellular carcinoma cell motility and invasiveness is associated with augmented metalloproteinase expression and secretion, together with down-regulation of E-cadherin (Cokakli, Erdal et al. 2009). Alternatively, re-expression of caveolin-1 in lung adenocarcinoma cells is sufficient to promote filopodia formation, cell migration and metastatic potential of these cells (Ho, Huang et al. 2002). Thus, as so often, the alterations observed due to the presence of caveolin-1 depend on the model under study. However, all the aforementioned observations have in common that caveolin-1 promotes traits associated with increased malignancy of cancer cells.
Successful tumor cell metastasis to distant sites requires the acquisition of multiple traits, including the ability to migrate. Interestingly, caveolin-1 is required for cell polarization and migration in two and three dimensions (Parat, Anand-Apte et al. 2003; Beardsley, Fang et al. 2005; Santilman, Baran et al. 2007; Yamaguchi, Takeo et al. 2009). Additionally, caveolin-1 regulates the small GTPases Rho and Rac, which are required for actin dynamics, cell polarization and directional migration (del Pozo, Balasubramanian et al. 2005; Grande-Garcia, Echarri et al. 2007; Grande-Garcia and del Pozo 2008). Furthermore, phosphorylation of caveolin-1 on tyrosine-14 favors cell migration (Grande-Garcia, Echarri et al. 2007) and anchorage-independent growth via the adaptor protein Grb7 (Lee, Volonte et al. 2000). Thus, in addition to its role as a tumor suppressor, caveolin-1 clearly also displays characteristics of a protein that promotes cell migration and metastasis.

Many explanations for such variations in function exist. One possibility is that the role of caveolin-1 depends on the cellular environment and that tumor suppressor activity is developed in systems where negative signalling occurs downstream of caveolin-1. Alternatively, when presence of the protein is associated with more aggressive tumor behavior, positive caveolin-1 mediated signalling is likely to be more important (reviewed in (Quest, Leyton et al. 2004; Quest, Gutierrez-Pajares et al. 2008)). A fundamental problem here is that direct comparisons are difficult, since these distinct characteristics were observed in different experimental settings and cell models. Ideally, to begin to test the aforementioned working hypothesis and identify molecular features of caveolin-1 that contribute to one or the other behavioral pattern, an approach that permits evaluation of these two characteristics in the same cell/animal model would be required. In this respect, some recent results from our laboratory suggest that melanomas may represent an interesting model.




| | Caveolin-1 expression | | | | | |
|---|---|---|---|---|---|---|
| Tissue | Normal | Cancer | Late Phase | knock-out mice* | Cell transfection** | Repression** |
| Lung | High | Low | Metastasis | Hyperplasia | Cell polarization and migration | ? |
| Breast | High | Low | Metastasis | Increased susceptibility to tumors | Resistance to anoikis, sensitization to Gefitinib | High sensitivity to chemotherapy, invadopodia |
| Colon | High | Low | Multidrug resistance, Metastasis | Increased susceptibility to tumors | ? | ? |
| Prostate | Low | High | Metastasis | ? | ? | ? |
| Melanoma | ? | ? | Metastasis | ? | ? | ? |

* *in vivo* model
** *in vitro* model

Fig. 4. Dual role of caveolin-1 in cancer: Representative profiles of caveolin-1 expression in different types of human cancer cells.

Two profiles of caveolin-1 expression appear to prevail in human cancer (Figure 4). In the first case, caveolin-1 expression is as follows: i) caveolin-1 is expressed in normal tissue. ii) During the development of cancer, caveolin-1 levels decline. iii) In later stages caveolin-1 re-expression occurs. Examples here include lung, colon and breast cancer. The second possibility is: i) Essentially, caveolin-1 is not expressed in normal tissue. ii) Caveolin-1 expression increases with tumor progression. The typical example here is prostate cancer. As, indicated, in many cancer cell types expression of caveolin-1 is associated with enhanced migratory capacity and multidrug resistance. How changes in caveolin-1 expression relate to melanoma development remain unclear. These characteristics are summarized again in the table (Figure 4). Additionally, the effects of loss of caveolin-1 expression in different tissues of knock-out mice are mentioned, as well as the consequences of either over-expressing or down-regulating caveolin-1 in tumor cells.

## 10. Caveolin-1 in melanoma

Relatively little information is available concerning the role of caveolin-1 in melanomas. Early reports focussed on studying the relationship between this protein and glycosphingolipids, which are important constituents of membrane microdomains referred to as "glycolipid-enriched membranes", "detergent-insoluble microdomains" (DIMs) or simply "membrane rafts" because of their flotation behaviour on sucrose gradients following centrifugation. Depending on whether or not caveolin-1 is associated with such membrane rafts, these may become detectable as 50-100 nm invaginations of the membrane called caveolae. Both membrane rafts and caveolae have received considerable attention due to their participation in a variety of cellular processes, including endocytosis, cholesterol transport, micro-organism infection and signal transduction. A detailed discussion of these aspects can be found elsewhere (Quest et al., 2004; 2008). Here, they are relevant because of their reported role in the regulation of signals associated with the development of malignant properties in cancer cells. For instance, in B16 mouse melanoma cells, a GM3-enriched membrane subdomain implicated in adhesion and migration was found to contain a number of relevant signaling molecules, including c-Src, FAK, and RhoA. This GM3 enriched "glycosphingolipid signaling domain" however does not contain caveolin-1 and appears to represent a functionally distinct identity from caveolin-1 and cholesterol-enriched microdomains in these cells (Iwabuchi et al 1998).

Acidic glycosphingolipids also play an important role in tumor biology and particularly GD3 expression is increased in almost all malignant melanomas and melanoma cell lines. In human melanoma cells, GD3 presence is associated with enhanced tyrosine phosphorylation of the two adaptor molecules p130Cas and paxillin. In SK-MEL-28 human melanoma cells, GD3 accumulates at the leading edge together with the aforementioned adaptor molecules and is thought to promote migration. In cells expressing caveolin-1, GD3 becomes homogeneously distributed in the membrane, rather than concentrated in specific regions. Hence, caveolin-1 is suggested to function as a tumor suppressor in melanoma cells by disrupting GD3-mediated malignant signaling (Nakashima, Hamamura et al. 2007).

Alternatively, for a non-cutaneous, retinal melanoma, increased caveolin-1 expression was associated with enhanced malignancy. Previous reports had documented low levels of caveolin-1 expression in different cell types of normal murine retinal tissue. Essentially this was corroborated in human tissue and, additionally, the level of expression of caveolin-1

was shown to increase in retinal melanoma cells (Berta, Kiss et al. 2007). An even more recent study identified exosomes in the plasma of melanoma patients, with high levels of caveolin-1. Exosomes are small vesicle secreted by both normal and tumoral cells. In this particular case, they were attributed immunosuppressive effects and associated with malignant tumor progression (Logozzi, De Milito et al. 2009). This study suggests that mechanisms similar to those already mentioned for prostate cancer may be relevant to caveolin-1 function in melanoma metastasis.

The notion that caveolin-1 presence may favor metastasis is supported by additional studies. Felicetti and co-workers proposed that caveolin-1 expression is associated with increased metastatic potential in different human melanoma cell lines. Specifically, caveolin-1 expression was increased using retroviral vectors in human melanoma cell lines. For the WM983A melanoma cell line, caveolin-1 increased cell proliferation, anchorage-independent growth, migration and invasion. Also, caveolin-1 down-regulation in metastatic caveolin-1-expressing melanomas reduces their proliferation, as well as their tumorigenicity (Felicetti, Parolini et al. 2009). Rather surprisingly, another recent report indicates that caveolin-1 blocks metastasis of malignant melanomas in a murine model (Trimmer, Whitaker-Menezes et al. 2010). Caveolin-1 has previously been shown to down-regulate survivin expression by sequestering β-catenin to the plasma membrane and reducing β-catenin-Tcf/Lef dependent transcription (Torres, Tapia et al. 2006). To do so, cells must express E-cadherin. However, E-cadherin is frequently silenced in melanomas and downregulation of this protein is considered one of the possible causes of melanoma progression and metastasis. Indeed, B16F10 cells do not express E-cadherin. Accordingly, E-cadherin re-expression in B16F10 murine melanoma cell line restored the ability of caveolin-1 to reduce cell proliferation and increase apoptosis by repressing survivin (Torres, Tapia et al. 2007).

Once again these findings highlight the apparent ambiguity of caveolin-1 function in melanomas and clearly more studies are warranted. As mentioned previously, an ideal experimental system would permit observing the role of caveolin-1 as a tumor suppressor and promoter of metastasis in the same cell/animal model.

## 11. Tumor formation assays in mouse melanoma models

Currently available models for the study of melanomas include: i) Xeno-transplantation models using genetically modified animals. One frequently employed possibility here are immunosuppressed animals that can be challenged with cells of another species. (ii) Syngeneic transplantation models. In this case, melanoma cells are derived from the same host so that immunological responses are avoided. Some examples here include Harding-Passey melanoma in BALB/c x DBA/2F1 mice, the Cloudman S91 melanoma in DBA/2 mice and the B16 melanoma in C57BL/6 mice. (iii) Genetically modified animals that develop spontaneous melanomas. Examples here include mouse lines expressing known oncogenes, such as Ret or mutant forms of Ras and Raf under the control of ubiquitous or tissue-specific promoters. Such mice develop melanocytic hyperplasia, retinal pigmented epithelial tumors and melanomas (Becker, Houben et al. 2010).

### 11.1 Syngeneic B16-F10 murine melanoma model

In our laboratory, the effects of caveolin-1 have been evaluated using two *in vivo* model systems, a xeno-transplantation model for HT29 and DLD-1 human colon adenocarcinoma cell lines in nude mice (Bender, Reymond et al. 2000) and the syngeneic B16 murine

melanoma model in C57BL6 mice (Nicolson, Brunson et al. 1978). Both models serve well to assess the ability of a protein like caveolin-1 to function as a tumor suppressor or promoter upon subcutaneous injection of the tumor cells. However, a major advantage of the latter model is that the behaviour of cells injected into the tail vein can also be readily evaluated. Since these cells are pigmented, dissemination throughout the body or specifically to the lung can be quantified for cells independent of whether they have been selected for homing or not (B16F10 versus B16F0 cells). Additionally, this model is closer to the situation in human patients because the tumors develop from cells in syngeneic mice with a fully functional immune system.

The schematic below (Figure 5) summarizes the origin of B16F0 cells (poorly metastatic) and how B16F10 cells (highly metastatic) were obtained by repetitive cycles of injection into the tail vein and recovery from the lung. Moreover, the figure highlights how the behaviour of such cells can be evaluated in tumor formation and metastasis assays.

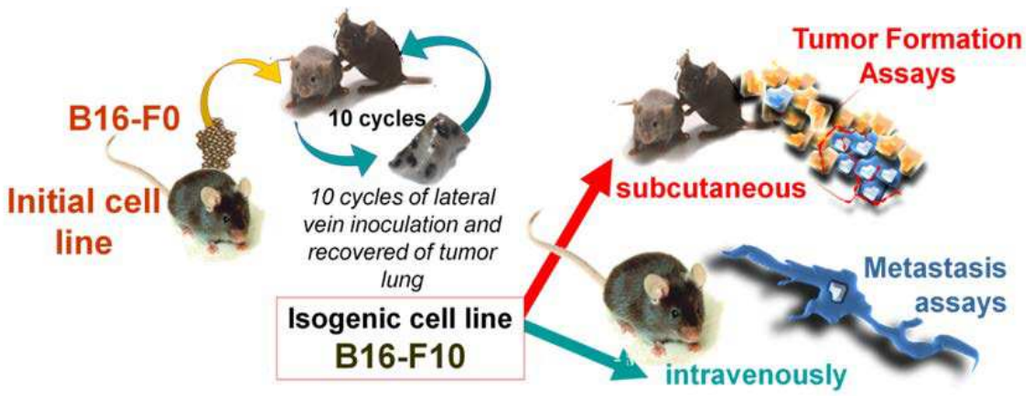



Fig. 5. Generation of B16F0 and B16-F10 cells (Nicolson, Brunson et al. 1978).

The parent tumor cell population of B16-F0 cells was isolated from a spontaneous melanoma in C57BL6 mice. These cells were then selected for their ability to colonize the lung following intravenous injection. To that end, B16-F0 cells were injected into the tail vein of C57BL/6 mice. Cells that formed tumors in the lung were expanded in culture and then injected again into the tail vein. This process was repeated ten times and the resulting population is referred to as B16-F10 cells. These cells behave very differently depending on the microenvironment. When B16-F10 cells are injected sub-cutaneously they form defined, palpable tumors. Alternatively, when injected intravenously these cells colonize the lung to form well defined black melanocytic nodules. See also figures 6 and 7, respectively.

### 11.2 Tumour formation in the B16F10 melanoma model

To study the ambiguity of caveolin-1 function, B16-F10 cells transfected or not with a plasmid (placIOP) permitting IPTG-inducible expression of caveolin-1 (plaIOP(cav-1)) were employed. These cells have been described previously (Torres, Tapia et al. 2007). The *in vivo* effect of the caveolin-1 expression in B16-F10 melanoma cells was evaluated by subcutaneous injection into of the flanks of mice. Appearance of tumors was monitored by palpitation and in initial experiments confirmed by histopathological analysis (Figure 6). Histological sections revealed that the tumors were composed entirely of proliferating

melanoma cells, whereas extracellular matrix, blood vessels and the stroma were largely confined to the tumor perifery. Hence, the tumors observed in this model system are rather soft and gelatinous, making manipulation for immunohistochemical analysis more difficult (Figure 6). Mice were monitored for 7 to 30 days after the challenge with tumor cells. As shown, our preliminary results using this syngeneic murine melanoma model indicated that caveolin-1 was able to suppress tumor formation *in vivo* (see Fig. 6A and 6B).

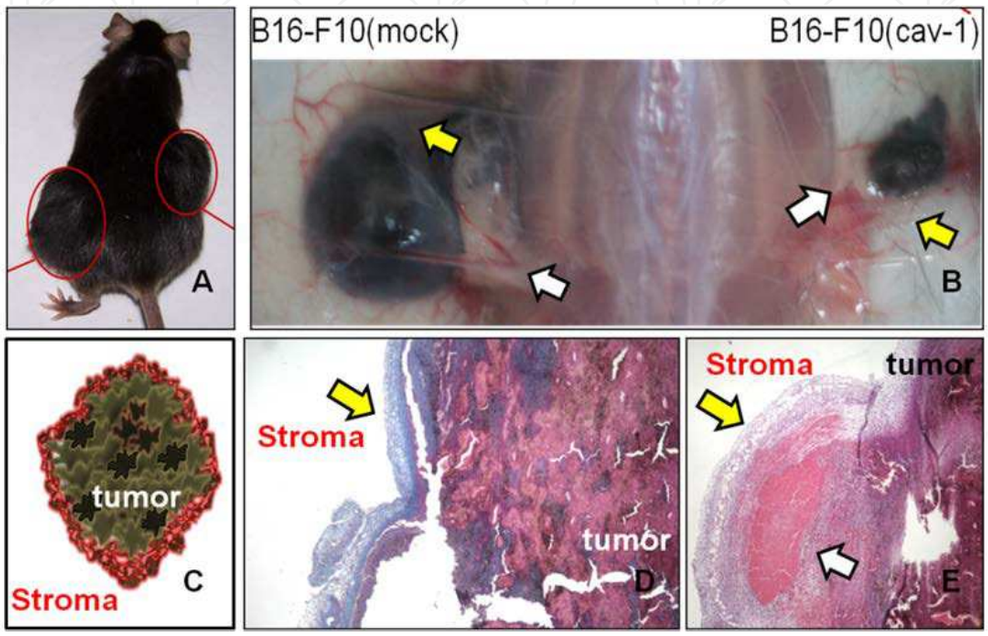



Fig. 6. Characterization of subcutaneous tumors.

C57BL/6 mice were inoculated with B16-F10 (mock) and B16-F10 (cav-1) cells on the left and right side of the animal, respectively. When the tumors of B16-F10(mock) cells reached the bioethically permitted limit the animals were sacrificed and the tumors were fixed in paraformaldehyde, embedded in paraffin, sectioned and stained with hematoxylin and eosin for histological analysis. A) Mouse inoculated with B16-F10(mock) and B16-F10(cav-1) cells injected into the left and right flank, respectively. B) Dissected tumors from mouse injected with cells as indicated in A. White arrows indicate external blood vessels. Yellow arrows highlight the tumor overlying serous tissue. C) Scheme showing how stroma support tissue surrounds the tumor. D) Histological section of a subcutaneous melanoma tumor, where the stroma and blood vessels surround the homogeneous tumor mass. E) Histological section of another subcutaneous tumor. White arrow: blood vessel located outside of the tumor; yellow arrow: stromal tissue.

## 12. Metastasis assays

To date, studies that employ the B16F10 model of lung metastasis generally quantify the metastastic nodules that are apparent at the lung surface (Figure 7). However, evidence in the literature indicates that this mode of quantification probably sub-estimates the actual extent of metastasis. Specifically, for B16F10 cells it is known that the cell adhesion

molecule Lu-ECAM-1 plays a fundamental role in retention of these cells in the lung. This molecule is present to some extent in venes of the parenchyma, but is strongly expressed particularly in sub-pleural and pleural venes, as well as in large venes of the lung and venes of the mesenchyma (Zhu, Cheng et al. 1991). The sites of expression Lu-ECAM-1 are entirely consistent with the distribution of melanomas we observed upon dissection of the lung. Moreover, the extent of lung metastasis observed by freezing the lungs of experimental animals, separating melanoma from normal tissue and then weighing both yielded results that were distinct from those obtained by simply counting nodules at the surface, as was reported (Trimmer, Whitaker-Menezes et al. 2010). In our hands, preliminary experiments indicated that the approach of actually quantifying lung tumor mass was far superior in detecting lung metastasis for B16 F10 cells expressing caveolin-1. Results obtained in this manner with B16-F10 cells clearly indicate that augmented caveolin-1 expression levels enhanced the metastatic potential of these melanoma cells when injected intravenously into animals. Taken together, the results discussed here suggest that the B16F10/C57BL6 model represents an ideal experimental system to identify molecular traits in caveolin-1 associated with its role as a tumor suppressor and promoter of metastasis.

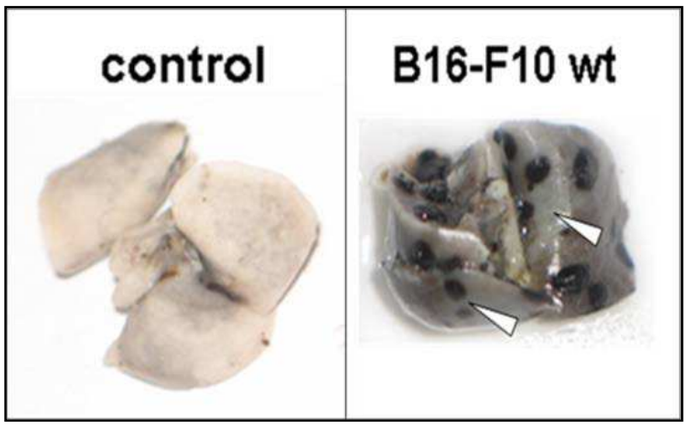



Fig. 7. Lung metastases.

B16-F10 cells were cultured in 100 mm plates for 48 h. Control mice were inoculated intravenously with saline solution (left panel) and the other group of mice were inoculated with 200,000 B16-F10 cells in 500 μl of saline solution (right panel). On day 21 post-injection, the animals were sacrificed, organs were removed and necropsied to identify melanocytic nodules. The lungs shown were fixed in Feketes buffer for 48 h. Arrowheads indicate nodules.

## 13. The caveolin-1 dilema: Summary and outlook

The data summarized here reinforce the notion that caveolin-1 potentially plays a dual role in melanoma development, as has been described for other cancers (reviewed in (Quest, Leyton et al. 2004; Quest, Gutierrez-Pajares et al. 2008)). The ambiguity of caveolin-1 function is perhaps best reconciled by the view portrayed in Figure 4. Colon cancer is an example where initial loss of caveolin-1 is followed by re-expression at later stages. If

caveolin-1 is re-expressed at early stages, it develops traits consistent with a role as a tumor suppressor. Regulation of the Wnt signaling pathway is one possibly relevant mechanism discussed here, although several more are likely to exist. During tumor progression, the cellular context changes, since expression of a large number of proteins is altered. One such possibility we eluded to is the loss of E-cadherin. However, extracellular components, such as $PGE_2$ are also relevant. Hence, both "intracellular" and also "extracellular" changes define caveolin-1 function in a cell. When then later in tumor progression caveolin-1 expression is triggered by as yet poorly defined mechanisms, the protein no longer encounters conditions that permit function as a tumor suppressor, and for instance suppression of β-catenin-dependent transcription. Instead, characteristics associated with malignant cell behavior, including increased cell migration, may prevail. For prostate cancer, the situation is different because caveolin-1 is not expressed in normal tissue. At this point, it is still not clear which of these examples is closest to the situation in melanomas. However, particularly the studies by Felicetti and co-workers (Felicetti, Parolini et al. 2009) indicate that prostate may represent a more appropriate comparison. However, it should be noted that loss of E-cadherin represents a crucial step towards metastasis in the development of melanomas. Bearing this in mind, one may suspect that augmented expression of caveolin-1 in later stages of melanoma development will promote the acquisition of a more malignant phenotype in these cells. The availability of an experimental model where both characteristics of the protein can be detected and analyzed *in vivo*, now permit defining the molecular traits associated with tumor suppression and enhanced metastasis. With such insight at hand, we may anticipate the design and use of successful caveolin-1-based strategies in the treatment of melanoma.

## 14. Acknowledgements

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## 15. Abbreviations

AA, arachidonic acid; APC, adenomatous polyposis coli; CSD, caveolin scaffolding domain; GSK3β, glycogen synthase kinase; IAP, inhibitor of apoptosis; MDR, multidrug resistance; NSAIDs, non-steroidal anti-inflammatory drugs; PCP, planar cell polarity; $PGE_2$, prostaglandin E2; $PGH_2$, prostaglandin H2; Tcf/Lef, T cell factor/lymphoid enhancer binding factor; SCF, Skp1-Cul1-F-box-protein.

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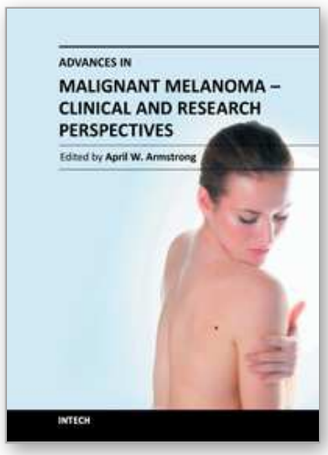


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This book titled Advances in Malignant Melanoma - Clinical and Research Perspectives represents an international effort to highlight advances in our understanding of malignant melanoma from both clinical and research perspectives. The authors for this book consist of an international group of recognized leaders in melanoma research and patient care, and they share their unique perspectives regarding melanoma epidemiology, risk factors, diagnostic and prognostic tools, phenotypes, treatment, and future research directions. The book is divide into four sections: (1) Epidemiology and Risk Factors of Melanoma, (2) Clinical Phenotypes of Melanoma, (3) Investigational Treatments for Melanoma and Pigmentary Disorders, and (4) Advances in Melanoma Translational Research. This book does not attempt to exhaustively cover all aspects of the aforementioned topics. Rather, it is a compilation of our authorsâ€™ pearls and unique perspectives on the relevant advances in melanoma during the recent years.

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