Molecular insights to a novel Caveolin-1-Rab5-Rac-1 signaling pathway important for metastatic cancer cell migration and invasion

Jorge Díaz1, 2, Natalia Díaz2, Lisette Leyton2, 3, Vicente A. Torres1, Andrew F.G. Quest2

1Institute for Research in Dental Sciences, Faculty of Dentistry, Universidad de Chile, Santiago, Chile
2Center for Molecular Studies of the Cell (CEMC), Advanced Center for Chronic Diseases (ACCDiS), Cell and Molecular Biology Program, Institute of Biomedical Sciences (ICBM), Chile
3Biomedical Neuroscience Institute (BNI), Faculty of Medicine, Universidad de Chile, Chile

Correspondence: Andrew F.G. Quest or Vicente A. Torres
E-mail: aquest@med.uchile.cl or vatorres@med.uchile.cl
Received: September 10, 2014
Published online: October 21, 2014

Caveolin-1 (CAV1) is a membrane protein that promotes Rac1 activation, migration and invasion of melanoma and metastatic breast cancer cells by mechanisms that have remained elusive. Recent evidence has shown that CAV1 sequesters p85α, a GAP for the small GTPase Rab5, thereby initiating a signaling cascade that leads to Rac1 activation. Rab5 has been identified as a critical regulator not only of early endosome dynamics, but also as an important regulator of cell adhesion and migration. Moreover, a novel pathway is described, whereby CAV1 stimulates the recruitment of Tiam1 to Rab5-positive early endosomes, leading to local Rac1 activation, migration and invasion of metastatic cancer cells.

Keywords: Caveolin-1; Rab5, Rac1; migration; invasion; metastasis

To cite this article: Jorge Díaz, et al. Molecular insights to a novel Caveolin-1-Rab5-Rac-1 signaling pathway important for metastatic cancer cell migration and invasion. Can Cell Microenviron 2014; 1: e332. doi: 10.14800/ccm.332.

Copyright: © 2014 The Authors. Licensed under a Creative Commons Attribution 4.0 International License which allows users including authors of articles to copy and redistribute the material in any medium or format, in addition to remix, transform, and build upon the material for any purpose, even commercially, as long as the author and original source are properly cited or credited.

The dual role of Caveolin-1 in cancer

Caveolin-1 (CAV1) is a membrane-associated protein that regulates numerous signaling cascades and plays a dual role in cancer development and progression. Specifically, CAV1 is thought to behave as a tumor suppressor during early cancer development and as a tumor promoter in advanced stages and metastasis [1-3]. CAV1 expression levels may vary dramatically depending on the model of study. Generally, a decrease in expression at early stages is associated with and thought to favor the development of lung, breast, colon and ovarian cancers as well as sarcomas and osteosarcomas. Moreover, re-expression of CAV1 in tumor cell lines is often sufficient to prevent tumor growth in orthotopic models [4-8]. However, despite such early loss, CAV1 is often found to be elevated again at later stages of many types of cancers. Also, in tissues lacking CAV1 expression, such as prostate and melanocytes, cancer progression is associated with enhanced CAV1 expression [9-12]. Importantly, such augmented expression is linked to increased tumor malignancy, poor patient prognosis, cell migration, multi-drug resistance and metastasis [1-3, 13-15].
Studies from our laboratory over the last years have attempted to understand this paradoxical scenario. Initially, we showed that CAV1 protein levels are decreased in both stroma and mucosa of colon tumors, relative to normal adjacent tissue. Moreover, a comparison of samples from different tumors revealed reduced CAV1 mRNA levels in colon tumors. Consistent with these findings, analysis of a variety of colon cancer cell lines uncovered low CAV1 mRNA and protein levels. Finally re-expression of CAV1 in these cells decreased tumor formation in nude mice. These findings are consistent with the idea that CAV1 participates as a tumor suppressor in colon cancer cells [6, 7].

Subsequently, we focused on identifying mechanisms to explain this role of CAV1. To do so, we initially asked whether proteins relevant to the genesis of colon cancer were affected by CAV1 presence and found that the inducible isoform of nitric oxide synthase (iNOS) was a downstream target of CAV1. Our studies demonstrated that the expression of CAV1 enhances iNOS degradation via the proteasome pathway [16, 17]. Afterwards, we took an unbiased approach and compared by microarray analysis how mRNAs from up to 20,000 genes varied in function of CAV1 expression in the colon cancer cell background. As expected, the expression of a large number of genes was altered. However, one of the most pronounced changes observed was for the inhibitor of apoptosis protein (IAP) survivin (unpublished data and [18]). The challenge then was to connect increased CAV1 presence in cells to reduced transcription of survivin. These efforts revealed that CAV1 inhibits β-catenin/Tcf-Dependent transcription of genes important for cancer cell survival, such as survivin [18], and also cyclo-oxygenase-2 (COX2) [19], by sequestering β-catenin to the plasma membrane. Most intriguingly, we found that CAV1 was only able to do so in cells expressing E-cadherin [18, 19]. In more recent experiments, we went on to demonstrate that these processes are also important in vivo. Using B16-F10 murine melanoma cells lacking both CAV1 and E-cadherin, we showed that expression of CAV1 reduced tumor formation in C57BL6 mice of subcutaneously injected cells, but promoted lung metastasis of the same cells when injected intravenously. Importantly, subcutaneous tumor growth was completely suppressed upon co-expression of CAV1 with E-cadherin. Also, transfection of cells with an E-cadherin encoding plasmid precluded the capacity of CAV1 to promote lung metastasis when cells were injected intravenously.
Comparable findings were obtained when A375 human melanoma cells were evaluated in B6Rag1−/− mice. Finally, the ability of CAV1 to promote metastasis in vivo was linked to activation of Rac1 [11, 12]. In summary, these studies identify how CAV1 functions as a tumor suppressor in a number of different types of tumor cell lines, including colon, breast, and melanoma cells, and importantly demonstrate that this mechanism is relevant in in vivo settings. Also, they revealed that enhanced CAV1 expression in the absence of E-cadherin promotes cell metastasis and that this ability correlates with enhanced Rac1 activity (reviewed in [3]). While a significant advance, what was still missing was a better understanding of how CAV1 connected to Rac1.

CAV1 in cancer cell migration

CAV1 was first identified as a highly tyrosine phosphorylated substrate in Rous sarcoma virus-transformed fibroblasts, which was indicative of a role for the protein in the transformation process [20]. Indeed, CAV1 phosphorylation on tyrosine-14 (Y14) favors anchorage-independent growth via Grb7 recruitment [5], integrin-dependent internalization of membrane microdomains [21], activation of matrix metalloproteinases and cell invasion [22]. Phosphorylation of CAV1 on Y14 is mediated by the non-receptor tyrosine kinases Src, Fyn and Abl following exposure to a variety of stimuli, including insulin, UV irradiation, hydrogen peroxide, hyperosmolarity and shear stress [23-26]. Importantly, CAV1 is known to increase polarization and directional migration in different cellular backgrounds [27, 28]. Accordingly, metastatic breast cancer cells (MDA-MB-231) express elevated endogenous levels of CAV1 and phosphorylation on Y14 is readily detectable. Moreover, shRNA-mediated down-regulation of CAV1 in MDA-MB-231 cells reduces velocity, directionality and persistency of migration [29, 30].

A considerable body of evidence implicates CAV1 in regulating the small GTPase RhoA, which modulates actin...
dynamics, cell polarization and directional migration [21, 31, 32]. Conversely, data obtained in metastatic cancer cells show that CAV1-enhanced migration and invasion is linked to the activation of Rac1 both in vitro and in vivo [11, 29]. The mechanisms underlying the CAV1-Rac1 connection were recently described [33] and these are discussed in the upcoming paragraphs.

The CAV1/p85α/Rab5/Tiam1/Rac1 axis in cell migration

As mentioned, the small GTPase Rac1 is a key element in the control of cell migration and several upstream regulators have been described [29, 33, 34]. Of particular interest in the following context is the signaling axis that controls local Rac1 activation and requires the early endosomal protein Rab5 [34].

Rab5 is a key regulator of the early endosome dynamics, implicated also in integrin trafficking and cell migration [35]. Recent research has shown that Rab5 activates Rac1 by recruiting the Rac1-Guanine Nucleotide Exchange Factor (GEF) Tiam1 to early endosomes and thereby promoting macropinocytosis of cell surface receptors and cell migration [34]. However, the upstream signaling events implicated in Rab5 activation and cell migration remained unclear. Based on data obtained in our laboratory, which showed that CAV1 promotes migration of metastatic cancer cells by increasing Rac1 activity and focal adhesion turnover [29], we hypothesized that a connection between CAV1 and Rab5 existed that was relevant to Rac1-enhanced migration of metastatic cancer cells.

Indeed, our recent studies identified a novel CAV1/p85α/Rab5/Tiam1/Rac1 signaling axis that is important in cell migration and invasion of metastatic cancer cells [33]. These studies showed that CAV1 induces Rab5-GTP loading and Rac1 activation, leading to increased cell migration and invasion in three different metastatic models: B16-F10 mouse melanoma, HT-29(US) human colon adenocarcinoma and MDA-MB-231 breast cancer cells. We also showed that CAV1 promotes recruitment of Tiam-1, a Rac1-GEF, to Rab5 positive early endosomes. Importantly, pharmacological inhibition of Tiam1 using the inhibitor NSC23766 revealed that Tiam1 is required for the CAV1-Rab5 axis to promote Rac1-GTP loading and cancer cell migration. Consistent with this interpretation, we also observed enhanced co-localization of Rab5 and Tiam1 on early endosomes. This suggests that the CAV1-Rab5 signaling axis promotes Rac1 activation by favoring recruitment of the involved components to the same membrane surface [33]. We then investigated whether the Rab5 GTPase-Activating Protein (GAP) p85α was involved in Rab5 activation induced by the presence of CAV1. Our findings revealed that p85α is sequestered in a protein complex with CAV1, preventing Rab5 inactivation, and thus enhancing Rac1 activity, migration and invasion.

In agreement with data showing that CAV1 promotes Rab5-GTP loading, additional studies indicate that morphological changes in the endosome population become evident when CAV1 is expressed (Figure 1). Specifically, the frequency of EEA1-positive early endosomes of different sizes was analyzed in B16-F10 cells either lacking or expressing CAV1. As shown in Figure 1, expression of CAV1 was associated with a higher percentage (34%) of large endosomes (0.5-2.0 μm²). These observations suggest that CAV1 promotes the accumulation of larger early endosomes, presumably by increasing Rab5-GTP levels, which is expected to increase endosome fusion [36-38]. However, in this scenario, we cannot exclude the possibility that CAV1 blocks -via an unknown mechanism- the early-to-late endosome maturation process. Additional studies are required to address this exciting possibility.

In summary, our recent findings uncover a novel CAV1/p85α/Rab5/Tiam1/Rac1 signaling axis. The salient features of this new CAV1-dependent signaling pathway that favors Rac1 activation, migration and invasion of cancer cells are summarized in the above scheme (Figure 2).

Conflict of interests

The authors declare no conflict of interests.

Acknowledgements

Fondecyt 1140907 and 11100287 (VT); CONICYT-FONDAP 15130011, Anillo ACT 1111, Fondecyt 1130250 (AFGQ); Fondecyt 1110149 and Iniciativas Científicas Milenio BNI P09-015-F (LL); Conicyt PhD Student Fellowship (JD, ND).

References

6. Bender FC, Reymond MA, Bron C, Quest AF. Caveolin-1 levels are down-regulated in human colon tumors, and ectopic


