Caveolin-1

A double-edged sword in the fight against cancer metastasis

- Professor Andrew Quest and his team at the Laboratory of Cellular Communication, University of Chile, have dedicated the last 25 years to uncovering the role of a protein called caveolin-1.
- Caveolin-1, or CAV1, has a highly unusual ability to promote cancer metastasis or suppress it, depending on the cancer type and stage.
- The researchers uncovered the underlying mechanisms of this protein and its interactions with other enzymes and molecular signalling pathways in cancer growth and cancer-fighting cell death.
- They found that phosphorylation of CAV1 promotes cancer metastasis, therefore inhibiting this process can help in the treatment of metastatic cancer.

Professor Andrew Quest and his team at the University of Chile have spent the last 25 years investigating the role of a protein called caveolin-1 (CAV1). CAV1 appears to have the unusual ability to act as both a tumour suppressor and an oncogene. The researchers have significantly improved our understanding of the role that CAV1 plays in different forms of cancers such as melanoma, breast, and colon cancer – rethinking conventional wisdom on cancer development in the process.

A double-edged sword

Cancer occurs when the normal systems that safeguard the body break down, thereby permitting unlimited proliferation of cancer cells and their dissemination to other sites of the body. This process is referred to as metastasis and is the leading cause of cancer-related deaths. Growth factor receptors responsible for stimulating cell proliferation and other signalling molecules can be upregulated or suffer gain-of-function mutations to become oncogenes that promote cancer (eg, *HER2* genes). In contrast, tumour suppressor genes that can 'switch off' the cell cycle and inhibit cancer (eg, *p53* genes) are either downregulated or suffer mutations that render them unable to stop the production of altered proteins, eventually leading to a tumour. Previously, it was widely believed that proteins could be either one or the other, a tumour suppressor or oncogene, but not both. However, the team's research upends this widely held assumption.

CAV1 is a structural protein that sits in the cell membrane where it has several functions including cell migration and signalling, cholesterol transport, and vesicle trafficking (transporting material inside or outside cells). The team uncovered the dual action of CAV1 at different stages of cancer progression to either switch off cancer cell proliferation or switch on metastasis.

Survivin' cell death

Quest and his team showed that CAV1 can act as a tumour suppressor by downregulating the apoptosis-inhibiting protein Survivin. Apoptosis is the programmed cell death mechanism in the body, which causes damaged or mutated cells to self-destruct. In cancer, the tumour cells proliferate unchecked by apoptosis, driving uncontrolled growth of these aberrant cells.

Survivin is highly expressed in many cancers; the protein is associated with chemotherapy-resistant cancer and indicates a poor patient prognosis. The Quest group also showed that it promotes tumour infiltration by blood vessel formation (angiogenesis) and inflammatory processes.

E-cadherin, CAV1, and metastasis

In a 2007 study using the HT29 human colon cancer cell line, the researchers linked CAV1's role as a tumour suppressor to the presence of E-cadherin. E-cadherin is a protein found on the surface of cells, where it regulates cell-to-cell interactions. Epithelial tissues make up the external layer of all body surfaces and E-cadherin is essential for binding epithelial cells together. Often this protein is lost in the process of cell transformation. Accordingly, the researchers found E-cadherin was not present in the same cell line at a later stage of cancer. Importantly, CAV1 only decreased Survivin in the cancer cells expressing E-cadherin.

E-cadherin and CAV1 have been observed to form a multi-protein complex that recruits β-catenin (a co-factor of transcription factors that regulate the expression of genes important in cell cycle, apoptosis, angiogenesis, and inflammation) to the plasma membrane, thereby decreasing the expression of Survivin. In doing so, apoptosis of cancer cells increases, while angiogenesis and inflammation decrease, which suppresses cancer growth.

Furthermore, in a paper published by Quest and his team in 2013, these findings were supported by an investigation into the interaction between E-cadherin and CAV1 in melanoma cells. They found that as levels of CAV1 and N-cadherin (a molecule that supports cell migration) were elevated for many of the metastatic melanoma cell lines, levels of E-cadherin were reduced. Using B16F10 murine melanoma cells expressing CAV1, they showed that in the presence of E-cadherin tumour growth was completely blocked, as was metastasis.

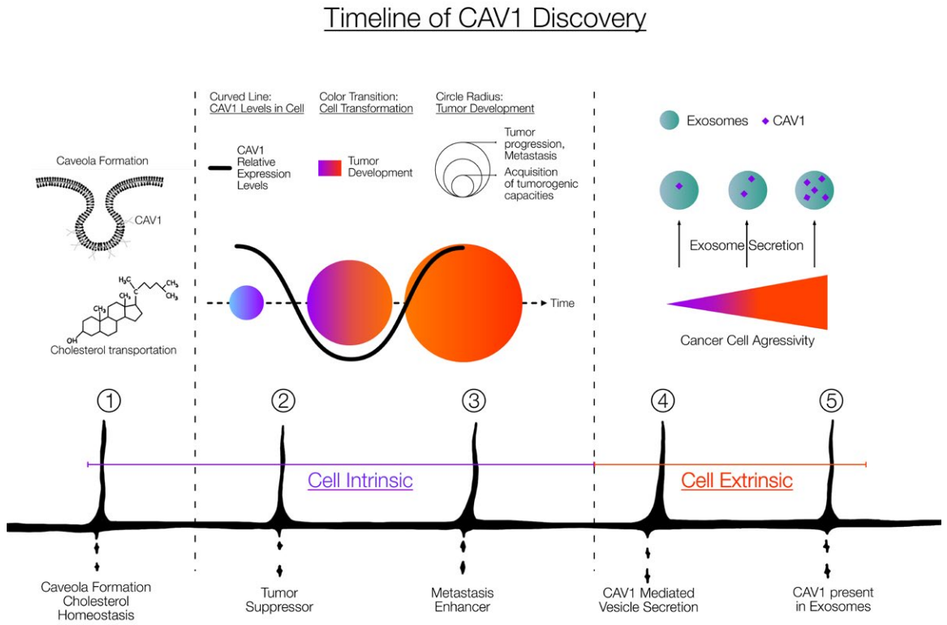
Although a drop in E-cadherin expression is observed during 'epithelial-mesenchymal transition' and in normal processes like wound healing, loss of E-cadherin is seen in many invasive cancers as its loss is critical to metastasis – allowing the cells to detach from the primary tumour and migrate to secondary sites. Increased CAV1 in the absence of E-cadherin was found to work as a 'molecular switch' that promotes metastasis.

> **CAV1 appears to have the unusual ability to act as both a tumour suppressor and an oncogene.**

In 2015, Quest and his group showed that the cancer-promoting traits were linked to the presence of CAV1 at or near the cell surface. An enzyme known as RAB5 that regulates intracellular vesicle trafficking was implicated in CAV1's cancer-promoting actions by activating the RAC1 signalling pathway involved in cell migration and cancer metastasis.

Blocking CAV1's pro-cancer action

In a number of studies from 2012 onwards, the group showed that the role of CAV1 in metastasis depends on its phosphorylation on the amino acid tyrosine-14, which depends on the activity of src family non-receptor tyrosine kinases. In a 2020 study, the researchers identified the mechanism by which phosphorylation at this site is reduced in the complex with E-cadherin. Phosphorylation is the addition of a phosphate group by enzymes called kinases to specific amino acids of proteins. Alternatively, phosphatases



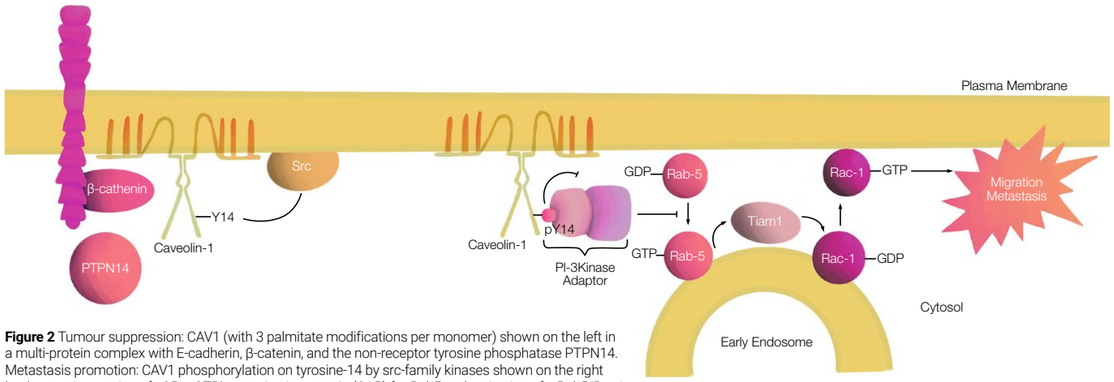


Figure 2 Tumour suppression: CAV1 (with 3 palmitate modifications per monomer) shown on the left in a multi-protein complex with E-cadherin, β-catenin, and the non-receptor tyrosine phosphatase PTPN14. Metastasis promotion: CAV1 phosphorylation on tyrosine-14 by src-family kinases shown on the right leads to sequestration of p85, a GTP'ase activating protein (GAP) for Rab5 and activation of a Rab5/Rac1 signalling cascade to promote metastasis. This sequence is blocked in cells expressing E-cadherin.

Figure 1
1) CAV1 was initially implicated in cholesterol transport and as a structural protein in caveolae.
2) CAV1 expression is lost in cell transformation and protein was implicated as a tumour suppressor.
3) In later stages of cancer, CAV1 expression increases again and enhances the acquisition of metastatic traits.
All characteristics highlighted so far are referred to as 'cell intrinsic'. 4) CAV1 was then identified as a secreted protein.
5) CAV1 identified in extracellular vesicles called exosomes and shown to be important in transferring malignant traits to other cells (cell extrinsic signalling).

remove phosphate groups in proteins, thus controlling their function. PTPN14 is a tyrosine phosphatase that has been previously implicated in controlling metastasis. It is found in the multi-protein CAV1/E-cadherin complex in cells expressing both proteins, resulting in reduced phosphorylation of CAV1 and is associated with reduced invasion of metastatic colon and breast cancer, as well as melanoma cell lines.

Metabolic switch in cancer development

For cancer cells to survive and multiply into a tumour, changes in cell metabolism are needed (known as the 'metabolic switch'), as the uncontrolled proliferation of cancer cells requires an extraordinary amount of energy in the form of glucose. In 2022, Quest and his team showed that CAV1 promotes this switch.

CAV1 appears to shut down the function of mitochondria, that is the oxygen-dependent energy production factory of cells. The cell under these conditions responds by switching to a glycolytic metabolism to compensate for the increased energy demands. The inhibition of mitochondrial respiration not only leads to higher glycolytic rates in cancer cells expressing CAV1 (and lacking E-cadherin), but also to increased production of oxygen radicals that block phosphatase function, elevate CAV1 tyrosine-14 phosphorylation, and promote the acquisition of more aggressive cancer cell traits.

New treatments on the horizon

The research into the pro- and anti-cancer ability of CAV1 and its synergy with E-cadherin, its functional interactions with RAB5, and the effect this has on cell signalling pathways such as those depending on RAC-1 creates a promising new avenue of treatment that can be of profound, life-saving benefit to patients suffering from highly aggressive, metastatic cancers.

Moving towards a translational approach, Quest has shown that inhibition of glycolytic reprogramming with antioxidants and pharmacological inhibition of CAV1 tyrosine-14 phosphorylation in CAV1 positive cells can prove efficient as a therapeutic means in the treatment of metastatic cancer.

> **Increased CAV1 in the absence of E-cadherin was found to work as a 'molecular switch' that promotes metastasis.**

Personal response

Did your findings showing that CAV1 is both a cancer oncogene and suppressor surprise you?

Although a rather unusual idea at the time, I was not surprised by our later findings. As discussed in the first paper we published on the role of CAV1 in cancer (*Cancer Research*, 2000), the pattern of CAV-1 expression can be distinguished in two phases during cancer progression. Downregulation of CAV-1 expression is required at the initial phase for primary tumor formation, while an increase of CAV-1 expression is observed as more aggressive cancer traits are accumulating. Similarly, increased expression of CAV-1 is also associated with metastasis.

Thus, although our entry to the field came with this initial publication on the role of CAV1 as a tumour suppressor, evidence available in the literature pointed towards a possible dual role. So, we actively pursued for the next approximately 20 years understanding this transition in function by first defining how CAV1 functioned as a tumour suppressor (downregulating genes like Survivin), then showing how it could promote metastasis (dependent on tyrosine-14 phosphorylation) and what this transition in function depended on (loss of E-cadherin).

How could your research contribute to the development of new treatments for cancer?

The simplest answer would be to knock out CAV1 in advanced-stage cancers. Alternatively, we need to prevent CAV1 phosphorylation on tyrosine-14 by re-expressing E-cadherin, or inhibiting the kinases that phosphorylate CAV1. All these approaches work in cells and in animal models. The problem, as for any treatment, is: how do you specifically target cancer cells? Potentially this may be possible using nanotechnology, which is something we have already started working on successfully. In 2018, we published a report showing that curcumin solubilised as a nanoemulsion was effective in preventing tumour reincidence and metastasis to the lung by melanoma cells in a mouse model (*Nanoscale* 2018). Another important point to mention here is that our nanoformulations of curcumin are now being commercialised as a nutritional supplement for humans and pets (see www.tiendananofix.cl).

What advice would you give an aspiring molecular cancer biologist who is interested in getting started in your field?

Cancer is an incredibly complex disease, largely because it is not just one disease, but rather a collection of diseases with several seemingly similar phenotypical manifestations. In fact, the definition of cancer is incredibly simple: 'Cancer is used as a term for diseases in which abnormal cells divide without control and can invade other tissues.' We know now that acquiring the traits necessary to divide and spread involves an enormous number of complex molecular mechanisms, attributable to both genetic and epigenetic changes, that may vary between cancers. So, questions to answer abound. The key is defining one that is transversal, relevant in different kinds of cancer and then doing good experiments. There is a considerable tendency these days to rely heavily on 'omics' approaches, which invariably yield a huge amount of data. I always tell the students in the laboratory that their hypothesis is one thing but the outcome of cleverly designed, well-done experiments, with the appropriate controls, is what will tell them where to go. So, my recommendation to an aspiring molecular cancer biologist would be to train him/herself in a broad variety of experimental techniques answering different biologically relevant questions (not necessarily cancer-related), working in different laboratories, preferably in different countries and then identify something he/she is specifically interested in and link it to a question of broader interest in the field.

What are your future research objectives?

In essence, the future has already begun with respect to the studies we are currently doing in dogs to evaluate the benefits of nanoemulsion application after removing tumours by surgery. Also, it remains to be seen whether in our experimental mouse model, we can prevent CAV1-enhanced metastasis of melanoma cells. More recently, we published a review article summarising how our view of what CAV1 does in cells has changed over time (*Biomolecules* 2019). As indicated by the diagram included here, our attention has recently turned to understanding cell extrinsic effects of CAV1, that is effects in other cells that essentially lack CAV1. We observed that aggressive breast cancer cells express high levels of CAV1 and liberate the protein in vesicles, called exosomes. Transfer of these vesicles to less aggressive cancer cells, renders the latter more aggressive (*Nanomedicine* 2018). We are also striving to understand the mechanisms by which CAV1 defines cargo composition of these vesicles. This may subsequently allow us to modify the composition and use such 'modified' vesicles for therapeutic purposes.

Details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Collaborators

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- Jorge Díaz, PhD
- Natalia Diaz, PhD
- Simon Guerrero, PhD
- America Campos, PhD

Bio

Andrew Quest, PhD is full professor at the University of Chile. Through his research, he aims to understand how changes in signalling processes contribute to cancer development and progression. In this context, the focus has been on understanding how the membrane protein Caveolin-1 can transition from beneficial acting as a tumour suppressor, to detrimental by favouring metastasis.

Lisette Leyton, PhD is full professor at the University of Chile. Her research focuses on how changes in signalling processes affect the behaviour of astrocytes and their interactions with neurons. Several of these mechanisms are also highly relevant in cancer.

Vicente Torres, PhD is full professor at the University of Chile. Vicente studies the role of small G-proteins in different types of cancer.

Lorena Lobos, PhD is associate professor, Universidad de Desarollo, Chile.
Major research focus is on the role of extracellular vesicles in the progression of breast cancer disease.

Further reading

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