THE IMPORTANCE OF S-PALMITOYLATION IN TUMORIGENESIS

Summary

What is S-Palmitoylation?
S-palmitoylation is a reversible post-translational protein modification, whereby palmitic acid forms a thioester bond with the sulphur atom of particular cysteine residues in target proteins.

How is it relevant to oncology?
Oncology includes the study of tumour formation. Dysregulation of S-palmitoylation affects cell homeostasis, and can produce tumors. Both insufficient and excessive S-palmitoylation can contribute to tumorigenesis and cell metastasis. A balance of S-palmitoylation and depalmitoylation thus must be achieved to maintain normal cell function.

What if it applies to my research?
The CAPTUREome™ S-Palmitoylated Protein Kit can be used to identify proteins modified with S-palmitic acid in your experimental system.

Read on to find out more!

1. DHHC Proteins & Tumorigenesis
Proteins with an Aspartate-Histidine-Histidine-Cysteine amino acid sequence are called DHHC domain proteins, and they catalyse the S-palmitoylation of other proteins within the cell. **Loss-of-function mutations in DHHC-domain proteins are linked to several types of cancers:** zinc finger DHHC-type 2 (ZDHHC2) expression was found to be significantly reduced in advanced liver and gastric cancer. Li et al.¹ (2014) found that 60% of their liver tissue samples taken from patients with hepatocellular carcinoma presented with low ZDHHC2 expression; this corresponds with Yan et al.² (2013)
findings, where 45% of their study presenting with gastric adenocarcinoma also exhibited significantly less ZDHHC2 expression as compared to adjacent normal tissue samples\(^2\)(p<0.05). Without ZDHHC2, proteins involved in cell fusion and adhesion are downregulated due to a lack of S-palmitoylation\(^3\), and since these cancers tend to present with a high degree of metastasis\(^4\), it can be concluded that ZDHHC2 is required to enhance cell-cell interactions through S-palmitoylation, and a reduced expression of this key enzyme contributes to the progression of cancer.

Conversely, other DHHC proteins associated with cancer, such as ZDHHC7 and ZDHHC21, appear to exacerbate the condition through S-palmitoylation. These enzymes act on sex steroid receptors to activate the MAP/ERK pathway of cell signalling (see Figure 2).

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Figure 2: Schematic diagram of the MAP/ERK pathway activated by fibroblast growth factor (FGF). Neural cell adhesion molecule (NCAM), usually expressed only in neurons, also activates the MAP/ERK pathway either through indirect stimulation of FGFR, or via the proto-oncogene protein Fyn (which in turn activates Ras to initiate the rest of the MAP/ERK pathway.) NCAM is expressed in certain ovarian carcinomas, where they lead to aggressive metastasis, and cancer proliferation is thought to do with activation of the MAP/ERK pathway (Turner & Grose, 2010\(^16\); Ditlevsen et al., 2007\(^15\)). S-palmitoylation of NCAM thus results in overstimulation of its expression on the membrane surface.
ZDHHC7 is thought to promote cancer progression via overactivation of the MAP/ERK pathway, as well as S-palmitoylation of the neural cell adhesion molecule, which is expressed in aggressive ovarian epithelial carcinomas. Estrogen receptors are upregulated in premalignant and malignant breast tumours, and this increase is associated with the upregulation of other sex steroid receptors which overactivate the MAP/ERK pathway. **Future strategies in treating breast cancer may look at targeting these DHHC-containing enzymes, or disrupting the S-palmitoylation process, in order to reduce cancer cell metastasis and proliferation**.

2. Ras Palmitoylation in Cancer

**Figure 3:**
**Diagram depicting Ras S-palmitoylation:**
(1) Prior to palmitoylation, Ras can interact with the Golgi and endoplasmic reticulum (ER) reversibly through transient membrane attachments.
(2) Palmitoyl acyltransferases (PATs) S-palmitoylates and traps Ras to these organelles, allowing for packaging and secretion of these proteins to take place.
(3) Ras is localized to where it is needed (e.g., the pathway in Figure 1).
(4) Upon activation, Ras is depalmitoylated by surrounding enzymes, allowing it to transiently return to the cytosol and re-enter the S-palmitoylation process. (Goodwin et al. 10)

S-palmitoylation facilitates cell signalling in the human body; hence, inappropriate S-palmitoylation would result in overactivation of the signalling processes, which can influence gene expression. Ras, found in all cell lineages, are highly involved in intracellular pathways that regulate cell proliferation; **palmitoylation and depalmitoylation of these proteins ensure correct localisation, and adjusts activation and inactivation of their signals** as well. The different Ras isoforms localise to distinct domains either via a Golgi-dependent or Golgi-independent pathway when S-palmitoylated (see Figure 3). **Inappropriate Ras activation due to defective depalmitoylation is present in up to 30% of cancer cases**, resulting in uncontrolled cell growth and proliferation.
Ras hyperactivation results in a chronic change in cell signalling, leading to cancerous cell progression. Depalmitoylation ensures that the Ras proteins are no longer effective, and are trafficked back to organelles for repalmitoylation or recycling\textsuperscript{10}.

Compartmentalisation of Ras proteins has also been proven to be essential in maintaining normal cell function\textsuperscript{11}; while S-palmitoylation serves to localise the proteins to regions of the membrane containing separate effectors and activators, other modifications are required to facilitate their movement to and from these regions. Rocks et al.\textsuperscript{12} (2005) conducted fluorescence studies to observe the movement of Ras proteins from Golgi to plasma membrane, and found that depalmitoylation aided primarily in retrograde trafficking from the membrane to the Golgi. This suggests that the reversibility of S-palmitoylation is crucial to maintaining normal cell functions, and any dysregulation results in tumorigenesis.

### 3. Apoptosis & Palmitoylation

Cell apoptosis is essential in regulating cell numbers and maintaining proper cell function. Receptors FasL, FasR and death receptor-4 form part of the death-inducing signalling complex (DISC) that activates this process; S-palmitoylation allows them to carry out their functions at the plasma membrane\textsuperscript{13}. In tumorigenesis, DISC is down-regulated due to an increase in depalmitoylation. FasR depalmitoylation is facilitated by acyl protein thioesterases 1 and 2; in Berg et al. (2015) study of chronic lymphocytic leukaemia (CLL) cells\textsuperscript{14}, a reduced DISC palmitoylation status was observed (p<0.02), and was associated with overexpression of thioesterases. This suggests that the depalmitoylation of these death receptors reduced cell sensitivity to external signaling, contributing to tumour growth and apoptosis resistance. This emphasizes the importance of S-palmitoylation in aiding normal cell signaling to prevent deviant cell growth.

In conclusion, a balance between S-palmitoylation and depalmitoylation is required to maintain normal cell function; any dysregulation that affects this process beyond normal homeostatic levels leads to tumorigenesis and aberrant cell proliferation, exemplified by the increased incidences of cancers associated with inappropriate amounts of S-palmitoylation. This reversible protein modification process is essential for cell–cell interactions, signalling pathway activation and protein compartmentalisation within the cell; without it, one can only imagine the disruptive – or even non-existent- signalling processes that will emerge from a state of no S-palmitoylation.
KEY POINTS

- Normal cell signaling and function is achieved by a balance of protein S-palmitoylation and depalmitoylation
- Loss-of-function mutations in DHHC-domains are linked to several types of cancer
- Inappropriate Ras activation due to dysregulated depalmitoylation is present in up to 30% of cancer cases
- S-palmitoylation regulates cell apoptosis both by protein activation and membrane localisation


