

Gene of the month: Cornulin

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ABSTRACT

Cornulin (*CRNN*) gene encodes a 495 amino acid long protein and is located on chromosome 1q21.3. Primarily, it functions as the marker of differentiation. Initially, it was found to be specific for the squamous cells of oesophagus. However, later on, several studies have revealed the presence of Cornulin downregulation in various epithelial squamous cell carcinomas of the head and neck, oesophagus and cervix and clinically associated it with worsening of cancer and the poor prognosis. Cornulin levels also showed dysregulation in other diseases such as Eczema and Psoriasis. Besides the differentiation marker, it was identified to be involved in the stress response. The studies, in psoriasis and oesophageal squamous cell carcinoma, has elucidated that the dysregulation in the Cornulin is associated with the cell cycle events such as G1/S transition. However, the actual function of Cornulin is still yet to be explored in detail.

INTRODUCTION

Cornulin gene and structure

The gene encoding Cornulin (*CRNN*) is present on the first chromosome at position 1q21.3 (also known as *c1orf10*). The different genes that function in epidermal differentiation span the 2Mb band region of chromosome 1q21, so this region is known as the epidermal differentiation complex region.¹ The epidermal differentiation complex region consists of genes of the three families clustered together. These genes encode (1) the structural proproteins such as involucrin, loricrin and small proline-rich proteins of the cornified cell envelope and late cornified envelope genes, (2) calcium-binding proteins such as S100 proteins, containing EF-hand domains and (3) the family of 'fused gene' proteins such as profilaggrin, trichohyalin, hornerin, repetin.² The *CRNN* gene consists of three exons interspersed by two long introns, the first exon being the non-coding while the second and third exons code for 495 amino acids long Cornulin protein (also known as squamous epithelial heat shock protein 53).³ The structural characteristics of Cornulin resemble the family of 'fused genes', that is, EF-hand domains at the N-terminus followed by multiple tandem peptide repeats. The N-terminal of Cornulin consists of an EF-hand calcium-binding motif of 90 amino acids as well as the conserved two repeated sequences of 60 amino acids^{3,4} (figure 1).

Functions of Cornulin

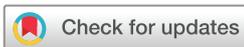
Primarily, Cornulin represents a squamous cell-specific protein due to its expression being squamous tissue-specific and is found to be expressed in squamous cells of oesophagus, cervical epithelium and skin.^{3,5} Cornulin as a member of the fused gene

family, localised in the epidermal differentiation complex region, is also characterised as a marker in epidermal differentiation (figures 2 and 3). N-terminal of Cornulin consists of an EF-hand calcium-binding motif of 90 amino acids, but the exact role that Cornulin performs after binding to calcium is yet to be explored.⁴ Nonetheless, the actual function of Cornulin is still largely unknown but is identified to be involved in the stress response. Cornulin also shows upregulation during deoxycholic stress and thereby preventing apoptosis in cells.⁶ Furthermore, in oesophageal squamous cell carcinoma Cornulin is shown to regulate the cell cycle arrest at the G1/S checkpoint by upregulating the expression of P21 and retinoblastoma, suggesting Cornulin to have a plausible tumour suppressive role.⁷ In contrast, the other study in psoriasis has shown results with regard to the regulation of the cell cycle by Cornulin. The study by Li *et al* demonstrated that Cornulin regulates the transition from the G1/S phase of the cell cycle by regulating the expression levels of cyclin D1. Cornulin overexpression upregulates the levels of Cyclin D1, thereby leading to cell proliferation⁸ (figure 4). The functions of Cornulin in these aspects need further exploration.

CORNULIN IN CANCER

The downregulation of tissue Cornulin was reported to be one of the prominent hallmarks in carcinomas of the oesophagus, cervix as well as head and neck.^{7,9-11} In a study by Xiao *et al*,¹² the quantitative proteomic analysis of the microdissected oral epithelium performed for the discovery of a biomarker for cancer, Cornulin was found to show the maximum decrease in the expression among 425 proteins quantified in oral epithelial dysplasia and oral squamous cell carcinoma.¹² Cornulin repression in the oesophageal cancer tissues was also found to be associated with an increased cancer aggressiveness leading to poor prognosis, advanced stage of the tumour, enhanced nodal metastasis and decreased overall survival rate^{7,13} (figure 2). Although the role of Cornulin in carcinogenesis is largely unknown, an *in vitro* study by Chen *et al*⁷ revealed the involvement of Cornulin in cell cycle arrest in oesophageal squamous cell carcinoma cell lines. In this study, higher levels of Cornulin were demonstrated to arrest the cell cycle at the G1/S phase transition, strongly suggesting its role in cancer progression.⁷ Hence, these findings indicate that Cornulin must be playing a significant role in squamous cell carcinoma pathogenesis.

Arnouk *et al* characterised tissue Cornulin as a biomarker in cancer such as cervical epithelial carcinomas due to its differential expression in



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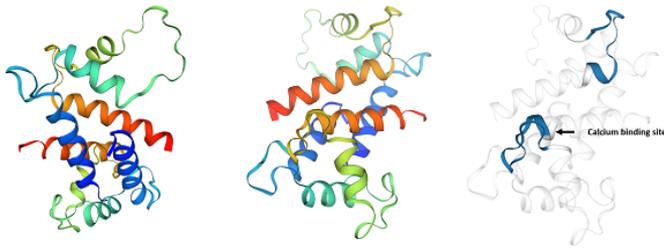


Figure 1 Structure of Cornulin: the structure of Cornulin is shown as predicted by UniProtKB. It has calcium-binding EF-hand domain of 90 amino acids at the N-terminus followed by multiple tandem peptide repeats. It also consists of the conserved two repeated sequence of 60 amino acids.

normal tissues as compared with high-grade squamous intraepithelial lesions, and invasive cervical cancer.¹⁰ Interestingly, Cornulin expression levels can also distinguish between low and high-grade dysplasia and cancerous lesion progression in oral cancer.¹⁴ Cornulin expression was found to be downregulated in the tongue squamous cell carcinoma and it was associated with the tumour grade and the nodal metastasis¹⁵ (figure 2). In a study conducted by our group, on Liquid Chromatography-Mass Spectrometry (LC-MS/MS) analysis of the saliva samples of Oral Squamous Cell Carcinoma (OSCC) patients, we found that the salivary Cornulin levels were ~10-fold downregulated in patients in comparison to controls (unpublished data) (figure 5). Thus, it can be said that the cancer cells express low levels of Cornulin as compared with the normal cells, but the exact reason is yet to be elucidated. Overall, it points to the fact that Cornulin may be another tumour suppressor gene, playing an important role in regulating the pathways that are associated with the development of cancer cell proliferation, migration, invasion and apoptosis. On the contrary, Cornulin can also be postulated as a protein that is downregulated by the cancer cells in response to oncogenic changes.

CORNULIN IN OTHER DISEASES

The dysregulation in the levels of cornulin is also reported in various skin diseases such as eczema and psoriasis. Eczema is a chronic relapsing inflammatory skin disease. The disease is characterised by the impeded epidermal differentiation and enhanced proliferation of keratinocytes, along with the increased apoptosis of keratinocytes.¹⁶ The levels of the Cornulin were downregulated

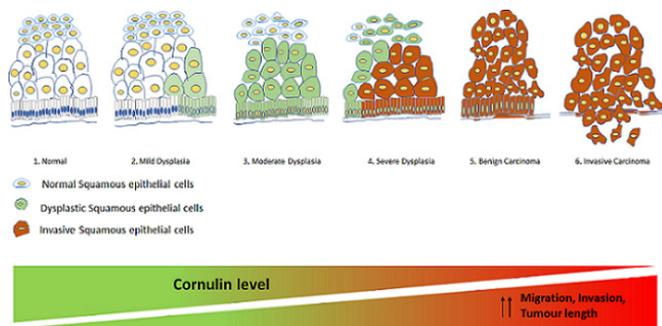


Figure 2 Cornulin level decreases with the progression of squamous cell cancers: the level of Cornulin decreases with increasing dedifferentiation of cells from normal cells to dysplasia and finally to invasive carcinoma. Decreasing levels of Cornulin are associated with more aggressiveness of the tumour (in terms of migration, invasion and tumour length).

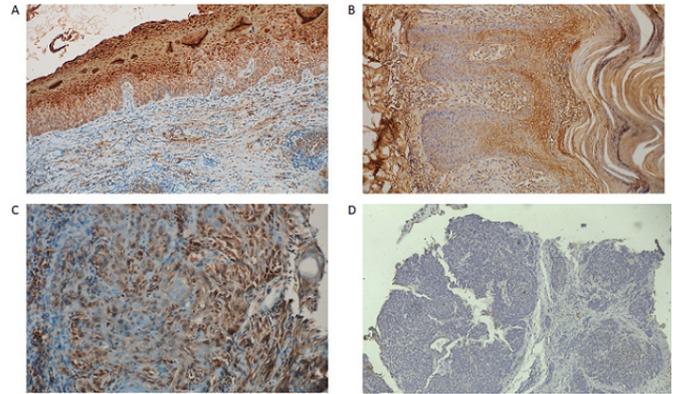


Figure 3 Immunohistochemical staining of human epithelial specimen for Cornulin expression in (A) normal squamous epithelial tissue (strong Cornulin staining $\times 20$). (B) psoriasis tissue (strong Cornulin staining in stratum spinosum $\times 40$). (C) well-differentiated squamous cell carcinoma tissue (moderate Cornulin expression $\times 4$). (D) poorly differentiated squamous cell carcinoma tissue (NO Cornulin expression $\times 10$).

in eczema. So the downregulation in the expression of Cornulin might be playing a role in decreased differentiation of keratinocytes contributing to the pathogenesis of the disease.

The expression of Cornulin was also dysregulated in psoriasis, a chronic inflammatory disease characterised by the hyperproliferation of differentiated keratinocytes. The expression of Cornulin was upregulated in the tissue of the psoriasis patients suggesting the fact that the upregulation in Cornulin levels might be contributing to the pathogenesis of Psoriasis⁸ (figure 3).

SIGNALLING PATHWAY FOR CORNULIN

The exact pathway in which Cornulin is playing the role is still unexplored. The different studies have revealed the dysregulation in the cell cycle genes due to the alteration in the levels of Cornulin protein. But still, an in-depth understanding is required to elucidate the role of Cornulin in the normal and the disease state.

Psoriasis is characterised by the hyperproliferation of basal keratinocytes and epidermal differentiation and is marked with the upregulation in the levels of Cornulin protein. The downregulation of the Cornulin levels in HEK293 and HaCaT cell lines resulted in the decreased proliferation due to the arrest in the

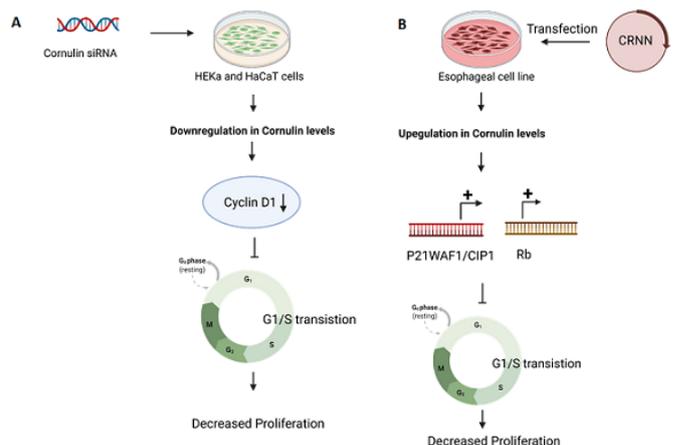


Figure 4 Signalling pathway for Cornulin as explored in (A) Psoriasis in (B) Oesophageal squamous cell carcinoma. The downstream effect of the dysregulation in the levels of Cornulin on the G1/S transition in case of (A) Psoriasis in (B) Oesophageal squamous cell carcinoma cell line.

MS/MS spectra for TMT labels

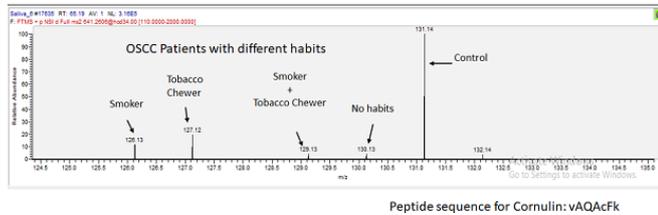


Figure 5 Salivary Cornulin level was downregulated by ~10-fold in the saliva of OSCC patients in comparison to the controls. LC-MS/MS spectra showed the downregulation in the levels of Cornulin in the saliva of OSCC patients in different cohorts (ie, smoker, tobacco chewer, smoker and tobacco chewer and NO habits) in comparison to controls. LC-MS/MS, Liquid Chromatography-Mass Spectrometry; NO habits, OSCC patients without any specific chewing or smoking habits; OSCC, Oral Squamous Cell Carcinoma.

cell cycle at G1/S transition along with the decrease in the levels of cyclin D1. Conversely, the upregulated levels of Cornulin led to increased cell proliferation, promoted G1/S transition and increased levels of cyclin D1. It was found that Cornulin increases cell proliferation by the PI3-Akt pathway.¹⁶

In contrary to the above mechanism, in the case of oesophageal squamous cell carcinoma, it was found that the Cornulin plays an important role as a tumour suppressor gene by arresting cell proliferation at G1/S transition by upregulating the levels of P21^{WAF1/CIP1} and retinoblastoma⁷ (figure 4).

PERSPECTIVE FOR CORNULIN DYSREGULATION IN CANCER

It has been reported in the literature that the levels of Cornulin are downregulated in the squamous cell carcinomas of the head and neck, oesophagus, and cervix. The decreased Cornulin levels were associated with the advanced stage of the tumour, enhanced nodal metastasis and decreased overall survival rate.^{7,13}

Cornulin has been proposed to be yet another tumour suppressor gene with an unknown mechanism of action. But it is not clear whether the loss of Cornulin is the cause of cancer development with the potential to transform a normal cell into a malignant cell.

In the case of lower expression of Cornulin being just a marker of cancer progression, it has the potential to predict the therapy outcome and clinical response in cancer of squamous cell origin.

So, in both the scenario (viz, Cornulin as a cause or as a biomarker), it is crucial to settle the ambiguity in this issue.

Take home messages

- ▶ Cornulin belongs to the family of epidermal differentiation complex region and is located on the chromosome at position 1q21.3.
- ▶ Cornulin is a member of the fused gene family, so it was also characterised as a marker in epidermal differentiation.
- ▶ Cornulin levels are downregulated in the squamous cell carcinomas of the head and neck, oesophagus, and cervix. Also, the decreased Cornulin levels were associated with the advanced stage of the tumour, enhanced nodal metastasis and decreased overall survival rate.
- ▶ Cornulin levels are downregulated in eczema while the expression of Cornulin is upregulated in psoriasis patients.

Although challenging, Cornulin either as a cause or as a biomarker will prove beneficial for the understanding of squamous cell carcinomas and clinical treatment outcome.

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