The Biological Role of Cervical Cancer Suppressor 3 (CCS-3): Literature Review

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Abstract: CCS-3 is one of the isoforms of eEF1A1, the key protein of the translation elongation step. Discovered in 2006, it has been the subject of some studies on human cervical cancer cell lines and cervical cancer samples. These studies brought to identify its probable role as a transcriptional co-repressor but other functions are currently unknown. It has also been shown that it is able to inhibit cell growth and induce apoptosis in the tumor cells that downregulate it. Further studies could highlight its functions and its possible usefulness as a tumor marker. This review aims to brief the research about CCS-3 that has been carried out so far.

Keywords: Cancer, EEFs, Cervical cancer suppressor 3 (CCS-3), EEF1A, Apoptosis.

INTRODUCTION

The eukaryotic translation elongation factors (EEFs) are a large protein family that plays a central role in the elongation step of translation. This family counts different proteins and their isoforms, conventionally divided into Non-Alpha eukaryotic translation elongation factors (Non-alpha EEFs) and Alpha eukaryotic translation elongation factors (Alpha EEFs). The eukaryotic translation elongation factor 1 alpha 1 (eEF1A1) is included in Alpha EEFs [1]. Its main isoforms are prostate tumor-inducing gene-1 (PTI-1)[2], more recently renamed eukaryotic translation elongation factor 1-alpha 1-like 14 (EEF1A1L14), a more basic isoform of eEF1A1 (MBI-eEF1A)[3], a cutaneous T-cell lymphoma (CTCL) antigen similar to eEF1A1, or HD-CL-08 [4], and cervical cancer suppressor 3 (CCS-3) [5].

The discovery of CCS-3 dates back to the year 2006 when Rho and colleagues [5] identified it as an isoform, or more correctly a splice variant, of EEF1A1.

This review work aims to summarize what has been discovered so far about CCS-3 as a starting point for further research.

GENOMICS

DNA Structure

The gene CCS-3 is one of the synonyms of EEF1A1 in GenBank database [6], in fact, as such it does not exist but its reference gene is EEF1A1. EEF1A1 is a coding gene of 5283 nt long (Ref. Seq. NC_000006) located on Chromosome 6 (6q13) with several alternative splicing transcript variants and protein isoforms [7]. CCS-3 is a splicing transcript variant of EEF1A1.

Pseudogenes

There are no known pseudogenes related to CCS-3, while for EEF1A1 there are currently 42 [7]. By aligning the nucleotide sequence of CCS-3 with the nucleotide sequences of pseudogenes of EEF1A1 it is clearly seen that CCS-3 is not derived from any of them, although for large section of nucleotide sequences, in particular for EEF1A1P5 and EEF1A1P6, there is an identity greater than 99% but less than 100% (*data not shown*). This high identity is normal since the EEF1A1 pseudogenes are derived from EEF1A1, probably through various types of mechanisms, but do not involve; and are not derived from CCS-3.

Transcriptomics

CCS-3 originates through alternative splicing of the EEF1A1 gene. An mRNA sequence for CCS-3 was deposited in GenBank with accession number AF322220. The sequence of full-lenght cDNA is 2979 nt long. The 5'-untranslated region (UTR) counts 1531 (1-1531) nt while the 3'-UTR counts 361 nt (2618-2979). The coding sequence (CDS) is 1086 nt long (1532-2617).

To date, there are no known non-coding RNAs (ncRNAs) sequences for CCS-3 nor miRNA or siRNA.

PROTEOMICS

Protein Structure

CCS-3 encodes a protein of 361 amino acids (Ref. Seq. AAN51932) with a molecular weight of 38.7 kDa

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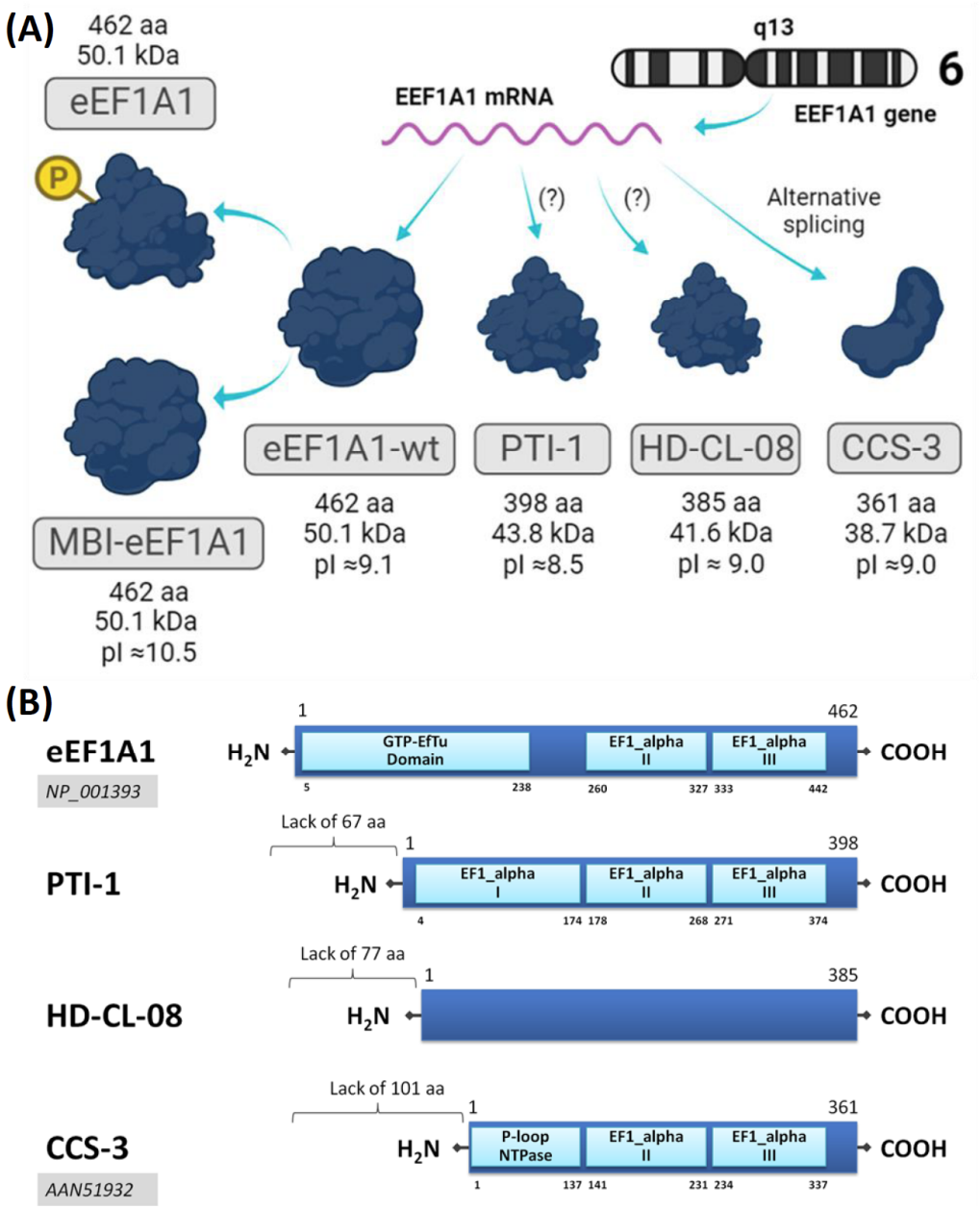

Figure 1: ***Homo sapiens*** **eEF1A1 and its isoforms.** The figure shows the wild type (wt) eEF1A1 protein and its currently known isoforms, i.e. MBI-eEF1A1, PTI-1, HD-CL-08, and CCS-3, which can be highlighted in western blot analysis [2,4,5,8]. The acronym eEF1A1 includes all forms of eEF1A1 with various post-translational modifications (such as phosphorylation, acetylation and others), which can be highlighted better in bidimensional electrophoresis gel analysis [3]. (**A**) Graphic representation of the various isoforms of eEF1A1 with indication of the main characteristics (created with BioRender.com); (**B**) Graphic representation of the various isoforms of eEF1A1 with evidence of the main domains and structural characteristics (reworked from [5], Pfam database and GenBank).

and a pI ≈9.0 (calculated with Compute pI/Mw tool by Expasy). Protein post-translational modifications, such as phosphorylation, have not yet been highlighted. The characteristics of CCS-3 and the similarities with EEF1A1, and the other isoforms of EEF1A1, are reported in Figure **1**.

CCS-3 shows 100% of homology and 100% of identity with EEF1A1 although its N-terminus shows a deletion of 101 amino acids compared to EEF1A1. No in-frame amino acid point mutations have been detected so far. Moreover, the three-dimensional structure of CSS-3 has so far not been investigated or published, while that of eEF1A1 is well known [9]. Here for the first time, in Figure **2**, a model of CCS-3 based on homology with eEF1A1 is proposed (calculated with Swiss-model tool by Expasy).

There is no known ortholog sequence for CCS-3 in other living animals or organisms.

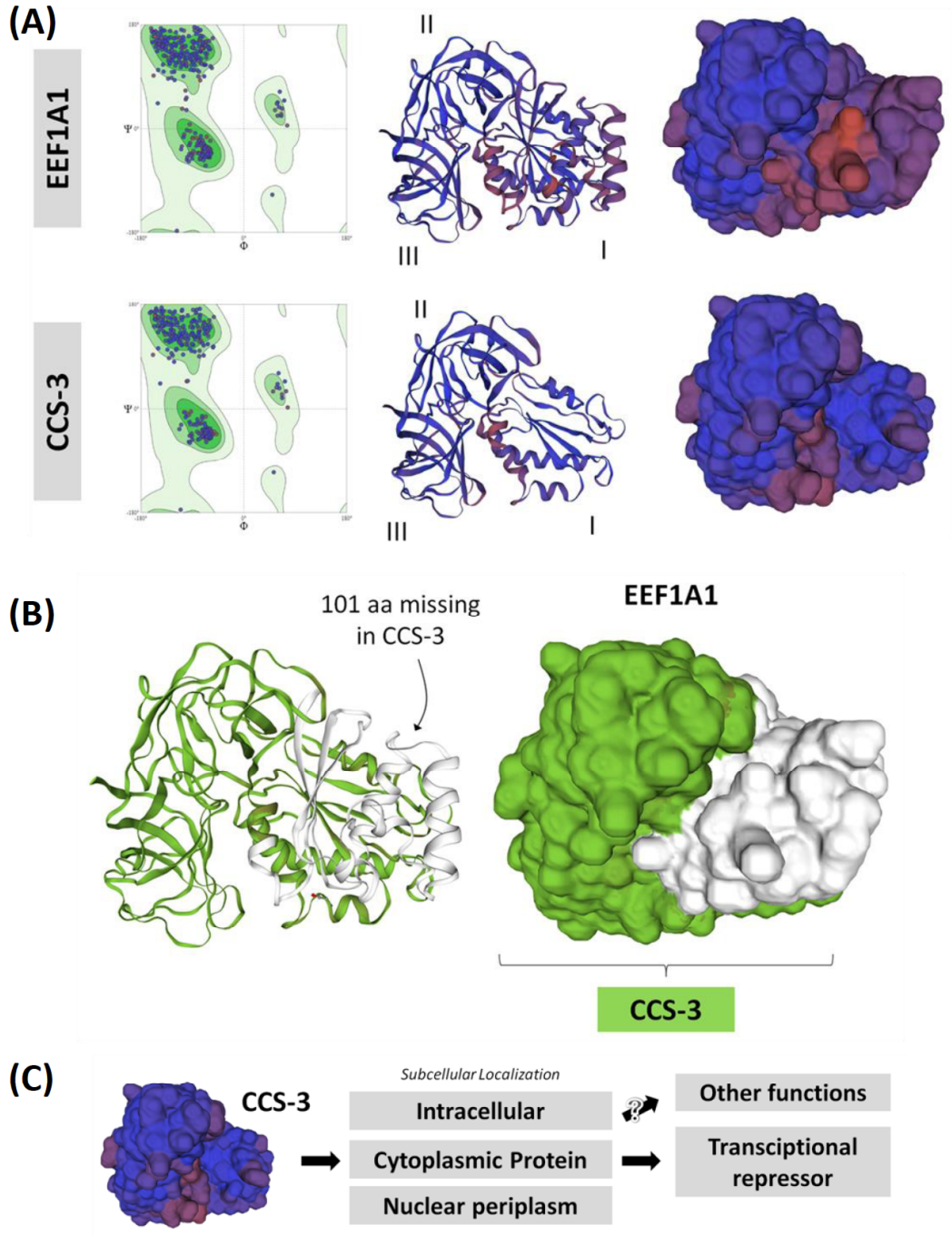

Figure 2: 3D structure model for CCS-3. The structural model for CCS-3 is conformed to the structure of eEF1A1 (created with Swiss-model). (**A**) Comparison between the 3D structure model of eEF1A1 and CCS-3 (*GMQE*: eEF1A1, 0,85; CCS-3, 0,88; *QMEANDisCo global score*: eEF1A1, 0,81 ± 0.05; CCS-3, 0,84 ± 0.05; *Ramachandran plot*: eEF1A1, 96,35%; CCS-3, 94,72%); (**B**) Evidence of the part of the protein missing in CCS-3 (white color) and, instead, present in EEF1A1. The loss of amino acids falls into the domain I of the protein; (**C**) Subcellular localization of CCS-3 as proposed by some authors [5,10] and predicted from PSLpred [11] and Protter [12].

Protein Functions and Localization

CCS-3, like eEF1A1, shows a mostly cytoplasmic localization. However, it also localizes in the nuclear periplasm [10]. It has been proposed that it acts as a transcriptional repressor [5]. A summary is shown in Figure **2C**.

Protein Interactions

CCS-3 interacts with promyelocytic leukemia zinc finger protein (PLZF) [5], proto-oncogene FBI-1 (Pokemon/ZBTB7A)[10] and other transcriptional co-repressors such as SMRT and BCoR [10]. Compared to eEF1A1, no other interactions of CCS-3 are known,

in particular with the cytoskeleton or with other elongation factors of protein synthesis.

CCS-3 IN CANCER AND OTHER HUMAN DISEASES

CCS-3 was found downregulated in human cervical cancer cell lines and in human cervical cancers (7 out of 8 samples, i.e. 87.5% of the samples) compared with normal human cell lines and tissues [5]. On the contrary, an overexpression of CCS-3 in cervical carcinoma cells inhibits cell growth by inducing apoptosis and suppresses the promoter activity of human cyclin A2 [5, 13]. Moreover, CCS-3 enhances transcriptional repression of the p21CIP1 gene (p21) by its interaction with FBI-1 [10].

eEF1A1 exhibits various functions within the cell, called moonlight functions, including cytoskeleton remodelling [14], promotion of misfolded protein degradation [15], control of the cell cycle [16], and the promotion of apoptosis [16]. EEF1A1 is often amplified and overexpressed in cancers [16]. Since eEF1A1 and CCS-3 have different and opposite functions, although further research on the N-terminal region needs to be done, Rho and colleagues have suggested that many of the functions of eEF1A1 reside precisely in the part of the protein that is deleted in CCS-3 [5]. Therefore, CCS-3 would lose the functions of eEF1A1.

Based on what has been observed so far, it could be concluded that CCS-3 in tumors functions as a transcriptional repressor and has an anti-tumor activity [5]. However, its role needs to be further analyzed in human cancers other than cervical cancer in order to understand its usefulness as a marker for tumour diagnosis, prognosis or progression [17].

There are no studies of CCS-3 in other human diseases.

CONCLUSION

CCS-3 is an isoform of eEF1A1. eEF1A1 isoforms are known to have roles in the cell, particularly in pathological conditions such as cancer.

CCS-3, compared to eEF1A1, lacks 101 amino acids in the N-terminal domain and acts as a transcriptional repressor. Other functions are unknown. It is downregulated in human cervical cancer cell lines and cancer samples compared to normal ones and when it is overexpressed in these cells inhibits cell growth and induces apoptosis. CCS-3 may be important in cell differentiation, tumorigenesis and oncogenesis but further studies are needed.

DECLARATIONS

Conflict of Interest

The author declares that there is no conflict of interest.

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ABBREVIATIONS

CCS-3 = cervical cancer suppressor 3

EEF1A1 = eukaryotic translation elongation factor 1 alpha 1

EEF1A1L14 = eukaryotic translation elongation factor 1-alpha 1-like 14

EEFs = eukaryotic translation elongation factors

HD-CL-08 = cutaneous T-cell lymphoma antigen

MBI-eEF1A = more basic isoform of eEF1A1

PLZF = promyelocytic leukemia zinc finger protein

PTI-1 = prostate tumor-inducinge gene-1 (alias, EEF1A1L14)

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