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Muscle-Specific FXR1 Isoforms in Squamous Cell Cancer

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Abstract

The RNA-binding protein fragile-X mental retardation autosomal 1 (FXR1) is upregulated in head and neck squamous cell carcinomas (HNSCCs) and expressed as at least seven isoforms in humans. Only two of these isoforms are capable of binding to RNA containing G-quadruplex structures. We suggest that these unique isoforms play a role in the pathogenesis of HNSCC.

Isoforms of the FXR1 Protein

The fragile-X mental retardation autosomal 1 (FXR1) protein binds RNA using several recognized motifs, and functions as a factor for mRNA transport, stability, and translation in eukaryotes [1–3]. Among FXR1's motifs are two regions that are rich in arginine and glycine and form RGG boxes [4]. These RGG boxes recognize G-quadruplexes, a secondary structure of DNA and RNA that forms between four coordinated guanines. The *FXR1* gene in humans encodes for a protein for which there are seven confirmed isoforms. Unfortunately, there have been many names for the isoforms and exons of FXR1 reported, which make the nomenclature more complex. Besides, different laboratories have referred to the same isoform by different names [1,2]. Here, we will use the National Center for Biotechnology Information (NCBI) nomenclature for FXR1 isoforms and exons; all amino acid residue numbering is based on the longest confirmed isoform, X1.

Protein analyses suggest that the seven FXR1 isoforms result from combinations of skipping exons 13, 17, and 18; use of an alternative start site within exon 4; and a frameshift in exon 19 that leads to early truncation (Figure 1) [2,5]. Expression of FXR1 isoforms is tissue specific [1–3]; isoforms X1 and X3 are predominantly found in normal skeletal muscle [1], whereas they are not appreciably found in other tissues [1]. X1 and X3 are the only isoforms containing exon 17 (Figure 1). Coupling the unique presence of this exon within these two isoforms with their tissue specificity, we hypothesized that X1 and X3 play a specific role in RNA recognition. We further hypothesized that this specific RNA recognition of X1 and X3 stems from exon 17 and may play a role in carcinogenesis. This hypothesis was bolstered upon a review of the literature.

Exon 17 houses a peptide sequence that uniquely allows FXR1 to bind G-quadruplex structures within RNA and DNA [4]. Specifically, the presence of amino acids in exon 17

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enhances the ability of the RGG box to bind G-quadruplex structures [4]. Additional supporting evidence comes from patients suffering from facioscapulohumeral muscular dystrophy, whose X1 and X3 display rapid turnover [5]. This turnover at least partially accounts for the poor control over cell cycle arrest and subsequent lack of myoblast differentiation seen in these patients. The mechanism behind this stems from recognition of the 3'-untranslated region (3'-UTR) of *p21^{Cdkn1a/Cip1/Waf1}* mRNA by X1 and X3, which then leads to its degradation and translational suppression [6].

The literature is scarce on definitive mechanisms or cell signals that lead to selective splicing of FXR1 to form X1 and X3, but two recent findings shed light on how exon 17 is included or excluded during transcription [7,8]. The *RBFOX1* gene encodes for a protein that recognizes a (U)GCAUG stretch in regulated exons [7]. Knocking out *RBFOX1L*, the homologous gene in zebrafish, led to skipping of exon 17 in FXR1. Whether this process translates to humans is, however, unknown. Further, an additional protein, RBM24, whose protein expression levels are controlled by miR-222, may also be involved in the inclusion or exclusion of exon 17 during the transcription of FXR1 [8].

FXR1 Controls *p21^{Cdkn1a/Cip1/Waf1}* Expression

In a knockdown study, researchers discovered an increase in *p21^{Cdkn1a/Cip1/Waf1}* mRNA levels upon reducing the cellular concentration of FXR1 [6]. Follow-up studies confirmed that knockdown of *FXR1* led to an increase in cell cycle arrest (increased G₀/G₁); the percent increase in arrested cells was directly related to the percent increase in the *p21^{Cdkn1a/Cip1/Waf1}* protein. X1 and X3 were mostly responsible for the destabilization of *p21^{Cdkn1a/Cip1/Waf1}* mRNA levels. This destabilization was shown to occur via recognition of a secondary RNA structure known as a G-quadruplex motif in the C-terminal end of *p21^{Cdkn1a/Cip1/Waf1}*'s 3'-UTR by FXR1 [6].

A recent study gave further weight to the control of *p21^{Cdkn1a/Cip1/Waf1}* translation by FXR1 and highlighted its potential role as an oncogenic driver in head and neck squamous cell carcinoma (HNSCC) [9]. HNSCC patients overexpress FXR1 at the DNA, mRNA, and protein levels. This analysis provides support to the amplification of the 3q chromosome that houses the *FXR1* gene in other SCCs (Figure 1) [10,11]. Specifically, the data demonstrate a marked increase in the expression of genes located on the 3q arm of the chromosome, where *FXR1* is housed, and a marked decrease in expression of genes located on the 3p arm.

Further, knocking down FXR1 led to increased cellular senescence [9]. Double-stranded breaks from increased DNA damage proceeded activation of p53 and the induced senescent state. This finding was attributed to the propensity of FXR1 to recognize an mRNA G-quadruplex structure in the 3'-UTR of *p21^{Cdkn1a/Cip1/Waf1}* mRNA, which led to destabilization and decreased translation of the corresponding protein, similar to the previous findings in facioscapulohumeral muscular dystrophy [6]. Further, this publication showed that X1 and X3, previously considered muscle specific, were also in HNSCC cells [9]. The combined findings that X1 and X3 were present in HNSCC cells, along with the FXR1-mediated decrease in *p21^{Cdkn1a/Cip1/Waf1}*, paved a path for the HNSCC cells to avoid senescence and replicate unchecked [9].

Concluding Remarks and Unanswered Questions

Herein, we discuss recent publications surrounding the X1 and X3 isoforms of FXR1. Our hypothesis that these isoforms have the unique capacity to bind the G-quadruplex structures in the 3'-UTR of RNA was validated by at least two independent laboratories [1,9]. This capacity allows X1 and X3 to selectively control the translation of p21^{Cdkn1a/Cip1/Waf1} and leads to impaired cell cycle control that may be an underlying cause of certain types of SCCs such as HNSCC [9] and lung carcinoma [9]. In addition, we confirmed that the 3q amplicon region housing the *FXR1* gene is also amplified in HNSCC patients as compared to control (Figure 1), similar to data for lung SCC [10]. Building off our literature review and findings in The Cancer Genome Atlas, we attempted to see if there was an upregulation or noticeable shift in expression patterns for X1 and X3 in HNSCC tissues compared with healthy tissue. Unfortunately, the Xena browser [12] does not currently support the transcripts that encode for Isoforms X1 and X3. As such, a bioinformatics approach to determining if individual isoforms of FXR1 are more or less prominent in certain forms of cancer is currently hindered until the databases are updated with the knowledge that Isoforms X1 and X3 exist at both the transcript and protein level for FXR1. In light of this, studies on the protein expression of FXR1 using antibodies that bind to all isoforms of FXR1 will be required. Specifically, currently marketed antibodies that target amino acid sequences found on exons 5–12, such as 6BG10, should result in the ability to visualize all isoforms of FXR1 known to be translated in humans. These studies will be needed to determine if our hypothesis and corollary findings could potentially be the underlying carcinogenesis for individuals with certain types of SCC.

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Figure 1. Splice Variants and Chromosomal Location of FXR1 and 3q Amplification in HNSCC Patients.

(A) Graphical depiction of the known isoforms of FXR1 protein in humans. The *FXR1* gene located on 3q26.33 (vertical blue line). (B) The chromosomal depiction of Chromosome 3 based on NCBI's most up-to-date Ideogram (2014). (C) Heat map of Chromosome 3's band expression in the TCGA Head and Neck Cancer Dataset compared to control tissue. Data were derived from Xena browser and plotted using GraphPad Prism. Chromosomal bands in cartoon depiction (B) align with heat map segments (C). FXR1, fragile-X mental retardation autosomal 1; NCBI, National Center for Biotechnology Information.