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Understanding the Relevance of Tpm1$_k$ Expression – Past, Present and Future

Mini Review

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Muscle contraction is dependent upon a cooperative interaction between thick and thin filament sarcomeric proteins. Tropomyosins (TPM), together with troponin and actin, form the thin filaments. TPM interacts with actin and the troponin complex to regulate contractile activity. During muscle contraction, an increase of calcium (Ca^{2+}) in the myofilament space promotes binding of this Ca^{2+} to troponin C, which alters the conformational state of TPM and facilitates acto-myosin interactions. TPM are a family of highly conserved actin binding proteins, which are expressed in all eukaryotes from yeast to humans. In vertebrates, except for zebrafish, four tropomyosin genes TPM1, TPM2, TPM3, and TPM4 are known [1-6] whereas there are six TPM genes present in zebrafish [3]. Each TPM gene is known to produce multiple isoforms via alternative splicing. Of these, TPM1 is the most versatile generating at least 10 alternatively spliced transcript variants [1-8]. In vertebrates, the predominant striated muscle isoform of the TPM1 gene known as TPM1α, contains exon 1a, 2b, 3, 4, 5, 6b, 7, 8, 9a and 9b and encodes a protein of 284 amino acid residues [1-5]. We were the first to discover another striated muscle isoform of the TPM1 gene, designated as TPM1$_k$ in vertebrates also encoding a protein with 284 amino acids. TPM1$_k$ contains exon 2a in place of exon 2b as in TPM1α (Figure 1). We first identified this isoform in axolotl (amphibian) [9] followed by chicken (avian species) [10], various mammals including horse [11], mice [12], humans [13], and also in zebrafish [14]. In chicken, however, transcripts of both sarcomeric isoforms of the TPM1 gene are expressed in embryonic hearts but are absent in post hatched chicken hearts. It is still unknown whether TPM1 isoforms are essential for cardiac contractility in chicken embryos. Using our published protocol in embryonic axolotl heart, we are in the process of assessing the effect of isoform- specific sense and/or antisense oligonucleotides on cardiac contractility and myofibrillogenesis in embryonic chicken cardimyocytes [15]. The strategy for sense and anti-oligonucleotide treatment is shown in (Figure 2).

The expression of TPM1$_k$ transcripts in various animals varies from species to species. In axolotl, TPM1α and TPM1$_k$ transcript expression is comparable in heart and skeletal muscle (Table 1). In other mammals, except for humans, the expression level of TPM1$_k$ transcripts is very low compared to TPM1α, in both hearts and skeletal muscle. Compared with other species, the expression level of TPM1$_k$ is significantly higher in human hearts [12,15,16] except for the Mexican axolotl [17].

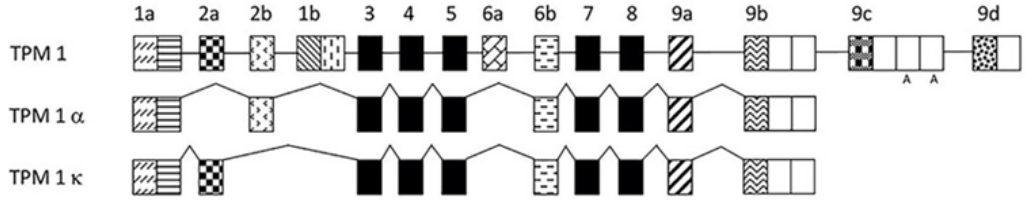

Figure 1: Two sarcomeric splice variants of the TPM1 gene.

Boxes represent exons and lines between two boxes represent introns.

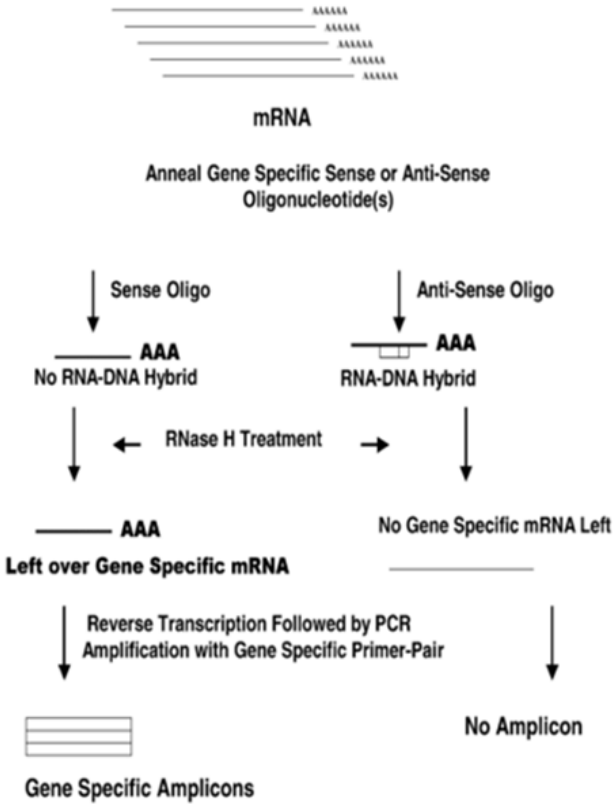

Figure 2: Strategy for determining the in vitro specificity of sense and anti-sense oligonucleotides.

TPM1k protein is expressed in human hearts as detected by western blot analysis with human-specific anti-peptide antibody [16] and by 2D western blotting with CH1 monoclonal antibodies followed by LC-MS/MS analysis [17,18], and by top down mass spectrometry [19]. The $TPM1_k$ protein has not been detected by either technique in human skeletal muscle. In human hearts the level of $TPM1_k$ protein expression is only ~5-6% of the total TPM protein [6,15,19,20]. In comparison, the $TPM1_k$ protein is expressed in both hearts and skeletal muscle in axolotl. Double labeled immunofluorescence experiments with anti-$TPM1_k$ antibody and MF20 (anti-Myosin) antibody, revealed the expression and incorporation of $TPM1_k$ protein in thin filaments of embryonic axolotl heart and skeletal muscle [18]. We also stained paraffin embedded tissue sections of human heart obtained at autopsy studies with human $TPM1_k$ anti-peptide antibody. Expression and incorporation of $TPM1_k$ into the myofibrillar bundles of the hypertrophied ventricular section was confirmed [18].

In order to explore the role of $TPM1_k$ in cardiac myofibrillogenesis and cardiac contractility, we blocked the expression of $TPM1_k$ in whole embryonic axolotl hearts by transfecting exon 2a-specific anti-sense oligonucleotides. RT-PCR analysis confirmed a lower level of $TPM1_k$ expression in the transfected axolotl hearts. Confocal microscopic analyses of the transfected hearts stained with anti-tropmyosin antibody showed lesser expression of the $TPM1_k$ isoform, which led to disrupted myofibril structure and lower contractility of the transfected hearts [15].

An increased level of $TPM1_k$ protein in human hearts of Dilated Cardiomyopathy (DCM) and Heart Failure (HF) patients was first reported by Rajan et al. [16]. In order to understand the role of $TPM1_k$ in mammalian myofibrillogenesis, they generated transgenic (TG) mice overexpressing $TPM1_k$ protein in a cardiac specific manner. None of the founder $TPM1_k$ mice nor their progeny demonstrated differences in either heart weight or in life span when compared with non-transgenic controls (NTG). An over-expression of TPM1κ concomitantly down regulates the expression of TPM1α so that the total sarcomeric TPM level remains constant. Histological analyses revealed no detectable changes in microscopic cellular morphology. However, echocardiographic analyses showed that mice overexpressing TPM1k had increased end-systolic and end-diastolic left ventricular dimensions. Biochemical and biophysical studies demonstrated less structural stability, weak actin-binding affinity and decreased Ca_{+2} sensitivity of TPM1k compared to TPM1α myofilament. Further studies are needed to better understand whether a small increase in $TPM1_k$ expression [21] observed in human heart failure patients represents a cause of cardiac dysfunction or a partial compensatory mechanism aimed at reducing the Ca_{+2} sensitivity of the thin filament toward the nonfailing state [20].

To have a clearer understanding of the role of $TPM1_k$ on cardiac contractility, one needs to also knock down the expression of $TPM1_k$ in human hearts/cardiomyocytes, which is a challenging job. As stated earlier, in the case of axolotl, it was done by transfecting antisense oligonucleotides. For human studies, we would like to exploit the use of cardiomyocytes derived from human inducible pluripotent stem cells (hiPSCs), which can be grown in monolayer culture [22]. Our preliminary results show very little expression of both TPM1α and $TPM1_k$ in uninduced stem cells compared to induced cardiomyocytes. It is to be noted that the ratio of TPM1α and $TPM1_k$ transcripts in the induced cardiomyocytes is quite comparable with what we found in human cardiac tissues. Our preliminary results on tropomyosin as well as other cardiac gene expression suggest that pluripotent cardiomyocytes will be an excellent model for studying the role of tropomyosin in cardiac contractility. Recently, using a RNA sequencing (RNA-seq) guides proteomics method, Lau et al. [23] reported two significantly regulated TPM1 isoforms expressed in day 7 hiPSC-CM and day h14 iPSC-CM. The first isoform was significantly down regulated in day 14 iPSC-CM and differs from the canonical TPM1α isoform by residues 189-212 (encoded by exon 6a) the accession number of which is P09493-4 in Swiss Protein data bases. The function of this TPM1 isoform in cardio-genesis is yet to be established. Interestingly, we cloned and sequenced cDNA of this isoform with RNA from human breast cancer tissues [8]. We called this isoform TPM1μ, the function of which is not known.

The second TPM1 isoform that was significantly upregulated in day 7 to day 14 hiPSC-CM was $TPM1_k$, which we also found (Unpublished results). Hence, in the future we will pursue our research on both $TPM1_k$ and TPM1μ, We would also like to explore whether an ectopic over-expression of TPM1k protein diminishes/inhibits the contractility of induced pluripotent human cardiomyocytes. For such analysis, we will transfect the pluripotent cardiomyocytes with the same $TPM1_k$ expression construct as used by Rajan et al to generate the transgenic mice [16].

Another observation that has drawn our attention is the absence of $TPM1_k$ protein expression in monkey hearts where only TPM1α protein is present [24 & our unpublished results]. As we stated earlier, $TPM1_k$ protein is expressed in human hearts. This disparity raises an important point/question on the evolution of the human heart. The genus Homo evolved ~2 million years ago and scientists have shown how drastically evolution has changed various organs like the brain and heart [25]. Shave et al. [25] reported extensive studies comparing the shape of hearts and various activities in chimpanzees, gorillas and humans. Although gorillas and chimpanzees spend a lot of time sleeping or being relatively inactive, they can be extremely active in short bursts of resistance physical activity (RPA) like climbing trees and fighting. These types of intense activities may create a pressure stress on the cardiovascular system. Monkeys may also follow a similar pattern of activities, whereas humans, during their early development spent a lot of time hunting, gathering, and later farming for their survival. In other words, humans, for their survival, depend on lifelong moderate-intensity endurance physical activity (EPA), which creates a cardiovascular volume stress. When left ventricular (LV) structure and function were compared, Shave et al. [25] showed that human LV possesses features that augment cardiac output, thereby enabling EPA. In addition, human LV demonstrate phenotypic plasticity as well as variability of various physical activities. These findings suggest functional differences between human and monkey hearts. Hence, it is arguably logical to detect differences in tropomyosin isoforms and other cardiac specific proteins expression in human and nonhuman primate hearts particularly of chimpanzee who are the closest living relatives of humans.

As can be seen in Table 1 detectable $TPM1_k$ protein expression in various vertebrate heart and skeletal muscle corelates with the level of mRNA expression. Some specimens showed no detectable 1 protein. This raises the question as to why are mRNAs for different sarcomeric TPM isoforms made at all, if the corresponding proteins are not required for various cardiac activities? Is it for emergency use if and when they are needed?

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