Abstract

Tbx20, a T-box transcription factor, is known to be crucial for cardio genesis and murine models with Tbx20 knockout fail to survive beyond E10.5. Tbx20 mutations correspond with severe congenital heart defects including valvulogenesis and septal defects. [1–3] All T-box proteins have a DNA binding domain. The T-box domain of Tbx20 consists of 180 amino acid residues and Tbx20 has a binding affinity to T/2 site [(**5'**-…AGGTGTGA…-3' over the consensus T-site 5'-…TCACACCT…-3'. [25] Tbx20 promotes cardiomyocyte proliferation via the Bmp2/pSmad1/5/8 and PI3K/AKT/GSK3β/β-catenin signaling pathways.[4] Tbx20 has also been found to act as a cardio-protectant against oxidative stress and downregulation of Tbx20 has been linked to increased apoptosis in cultured rat cardiomyocytes.[5] Further, the cardio-protective role of Tbx20 under ROS and hypoxic conditions in the H9c2 cell line was reported. [6] Tbx20 is known to interact with and induce other transcription factors like Nkx2.5 and Gata4 [7,8] which are also important for cardiogenesis, maintaining cardiac homeostasis and promoting the expression of Troponin-I and myosin heavy chain protein.

Autophagy is an evolutionarily conserved catabolic phenomenon that recycles cellular components to provide for bioenergetics and fuel for cellular survival under stress conditions. At the basal level, the goal of the autophagic machinery is to maintain cellular and organ homeostasis. This is achieved by providing catabolites like fatty acids and amino acids which in turn serve as substrates for many metabolic processes. The cargo (can be organelles, protein aggregates, lipids, cellular proteins) to be degraded is sent to lysosomes via autophagosomes whereby fusion of lysosomes with the latter forms autolysosomes and the cargo is thereafter degraded by lysosomal hydrolases. Autophagy can also be activated under stress conditions like nutrient scarcity/ caloric restriction, ROS

(Reactive Oxygen Species) accumulation, ER (Endoplasmic Reticulum) stress, and mitochondrial damage where autophagy serves as a substrate recycling machinery remove protein aggregates and provides much-needed ATP for the survival of cells. [9–11]Autophagy has been known to be a critical factor in the survival of neonates postpartum wherein it was found that Atg5 knockout mice models failed to survive after birth. Furthermore, in mice that survived the brief period of nutrient deprivation postpartum (which is the usual scenario), massive upregulation of cardiac autophagy was observed giving away the importance of autophagy in pro-survival while also bringing cardiac autophagy to the centre.[12] Impaired and altered autophagy has been an underlying cause of cardiac diseases like AMI (acute myocardial infarction), Ischemic heart disease (IHD) and cardiomyopathy. A similar role of autophagy has been found in acute myocardial infarction wherein inhibition of autophagy enlarges the infarct zone and decreases the ATP content of cardiomyocytes and there have been theories that augmenting autophagy would be a therapeutic approach in restoring the cellular integrity and cardiac functioning in these case[13–17] Aging is perhaps one of the greatest risk factors responsible for failing hearts. In fact, aged individuals with no underlying cardiac condition show poor cardiac functionality, diastolic function and left ventricular dilatation. [18–20]Accumulation of protein aggregates, misfolded proteins, a poor balance between ROS and anti-oxidants, mitochondrial derangements, attenuated expression of Sirtuins (a class of NAD^+ dependent deacetylating enzymes) especially Sirtuin 1, 3 (Sirt 1,3), GSk-3β contribute to cardiac aging.[21–23] As such, impaired and poor levels of autophagy are prevalent in aging hearts while apoptotic levels are on a surge. Augmenting autophagy by either calorific restriction or Rapamycin (Rap) administration has shown improvement in cardiac functioning and improved life longevity.[24–26]

Here in this study for the first time, we demonstrate the role of Tbx20 as a potential candidate to induce anti-senescence-like characteristics in the aging mice population. Autophagy induced expression of Tbx20 activates GSK-3β and

transcription factors Nkx2.5, Gata4 and Sirt1 after subjection to starvation (Strv) and rapamycin (Rap) treatment in both *in-vivo* (BALB/c mice) and *in-vitro* (H9c2 cell line) model systems. The upregulation of Nkx2.5 and Gata4 following autophagy induction is indicative of progression towards progenitor like cardiomyocyte characteristics, while activation of Sirt1 and GSK3β suggests an anti-aging/ senescence since Sirtuin1 is closely linked with aging, is known to be a mediator of caloric restriction and Sirt1 transgenic mice prevent early mortality. With pre-existing knowledge of the expression of Sirt1 and GSK-3β, under autophagy conditions along with the cardioprotective roles they play, these two were chosen as possible candidates that could interact with Tbx20. Further, Tbx20 loss of function (LOF) assay in the H9c2 cell line validated Tbx20-dependent expression of Sirt1, GSK-3β, Nkx2.5 and Gata4.

On the other hand, ECM remodeling in heart or cardiac remodeling remains an important factor in the pathophysiology of cardiovascular diseases.[27,28] Collagen-I forms the major component of the matrix interstitium of the myocardium in addition to Collagen-III, fibronectin, proteoglycans, tissue inhibitors of matrix metalloproteinases (TIMPs) and matrix metalloproteinases (MMPs). The three stages of cardiac remodeling following cardiac injury are inflammatory, proliferative and maturation phases leading to a mature scar formation.[29] The preliminary stages of ECM remodeling are necessary as it prevents rupture of the ventricular wall, however, exacerbated ECM remodeling leads to progressive fibrosis in the heart and cardiac malfunctioning.[30,31] The MMPs (zinc-dependent proteases) are involved in the turnover of matrix proteins like Collagen.[32] Adamts4, a member of Adamts family is an important MMP. Adamts4, also is a disintegrin with thrombospondin like motifs.[33,34] The mode of action of Adamts4 is by binding to ECM proteins and thereafter cleaving ECM proteoglycans like aagrecan, versican, brevican in addition to regulating Collagen turnover.[35,36] Adamts4 modulates the pathophysiology of osteoarthritis through degradation of

matrix proteoglycans and eventually lead to cartilage degradation which manifests as degenerative osteoarthritis.[37,38] Besides osteoarthritis, Adamts4, has also been linked with cancer and angiogenesis where its role remains controversial. Some studies report it to be an indicator of early-stage cancer like in cases of colorectal cancer, others findings suggest that its mutated and truncated fragments may suppress tumour growth through inhibition of angiogenesis. [39,40] However, the involvement of Adamts4 in cardiac remodeling is relatively less known. Only a few studies have shown the involvement of Adamts4 in atherosclerotic plaque development.[41] and recent studies have shown elevated expression of both Adamts4 and Adamts1 in patients with acute aortic dissection and coronary artery disease.[42,43] To decipher the molecular cascade of Adamts4 induction and associated signaling pathway, cultured H9c2 cells were used for *in vitro* experiments. Adamts4 expression was induced in H9c2 cells following hypoxia (Hyp) and ROS and Hyperglycaemic stress inductions. Additionally, Adamts4 expression was manipulated by siRNA-mediated loss of function and TGF-β inhibitor studies with SB431542/ALKI treatment *in-vitro* to evaluate the hierarchy and dependency on TGF-β signaling. TGF-β is a known marker for inflammatory and fibrotic responses following pathological stress like Myocardial Infarction, ischemia and reperfusion (I/R) injury.[44–48] Ultimately, ADAMTS4 expression was also assessed in patients with cardiac diseases namely Dilated Cardiomyopathy (DCM) and MI.

Overall, this study focusses on the role of Tbx20 under stress conditions and ECM remodeling.