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AGE-RELATED MORPHOLOGICAL REMODELING OF CARDIAC MUSCLE FIBERS AND CAPILLARY NETWORKS: A COMPARATIVE HISTOLOGICAL STUDY

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Abstract: *Aging exerts a profound influence on cardiovascular morphology and function. Cardiac aging is characterized by structural remodeling, including cardiomyocyte hypertrophy, interstitial fibrosis, and changes in microvascular architecture. This comparative histological study investigates age-related morphological alterations in cardiac muscle fibers and capillary networks using juvenile, adult, and aged rat models. Using Hematoxylin and Eosin, Masson's Trichrome, and CD31 immunostaining, we quantified changes in myocyte diameter, collagen deposition, capillary density, and vascular-to-myocyte ratios. Our findings demonstrate progressive cellular hypertrophy, increased fibrotic remodeling, and significant capillary rarefaction with aging. These histological changes have critical implications for age-related cardiac dysfunction and highlight the importance of vascular preservation in mitigating myocardial degeneration.*

1. Introduction

Aging is a complex biological process that affects all organs, with the heart being particularly susceptible due to its high metabolic demands and limited regenerative capacity. The aged heart exhibits a constellation of structural and functional changes collectively termed as 'cardiac remodeling.' These alterations, though adaptive initially, eventually predispose the heart to dysfunction and disease. Cardiomyocyte hypertrophy is a hallmark of cardiac aging. While increased myocyte size can maintain contractile force in the face of declining myocyte number, it is often accompanied by cytoplasmic vacuolation, nuclear enlargement, and mitochondrial damage (Olivetti et al., 1991). Simultaneously, there is enhanced deposition of

collagen fibers within the interstitium, leading to myocardial stiffness, impaired compliance, and increased risk of arrhythmias (Biernacka & Frangogiannis, 2011). Microvascular aging further exacerbates cardiac dysfunction. Age-related capillary rarefaction results in inadequate perfusion and oxygen delivery to hypertrophied myocytes, contributing to myocardial ischemia and metabolic stress (Tomanek, 2005). However, comprehensive comparative histological studies delineating these age-associated changes remain limited. This study aims to characterize and quantify histological changes in cardiac muscle and capillary structure across different ages using standardized staining and morphometric techniques, thereby enhancing our understanding of cardiac aging mechanisms and their potential therapeutic targets.

2. Materials and Methods 2.1. Experimental Design and Animal Models Eighteen male Wistar rats were divided into three groups based on age: juvenile (1 month), adult (6 months), and aged (24 months). All procedures conformed to institutional ethical guidelines for animal experimentation.

2.2. Tissue Processing and Histological Staining

Hearts were harvested post-euthanasia, washed with phosphate-buffered saline (PBS), and fixed in 10% formalin for 48 hours. Following paraffin embedding, transverse sections (5 μm) were stained with:

- Hematoxylin and Eosin (H&E) for general morphology
- Masson’s Trichrome for collagen/fibrosis quantification
- CD31 immunohistochemistry to visualize endothelial capillaries

2.3. Immunohistochemistry Protocol

CD31 antigen retrieval was performed in citrate buffer (pH 6.0), followed by blocking in 5% goat serum. Primary antibody (anti-CD31, 1:100) incubation was done overnight at 4°C, followed by HRP-conjugated secondary antibody and DAB visualization. Slides were counterstained with hematoxylin.

2.4. Morphometric Analysis

Images were acquired using a Leica microscope at 400× magnification. Ten randomly selected fields from left ventricular myocardium were analyzed per sample. The following parameters were evaluated:

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- Cardiomyocyte diameter (μm)
- Interstitial fibrosis area (% of field)
- Capillary density (capillaries/mm²)
- Capillary-to-myocyte ratio

2.5. Statistical Analysis

Mean values ± standard deviation (SD) were calculated. Differences among groups were assessed using one-way ANOVA and Tukey's post hoc test. Pearson correlation was used to evaluate the relationship between capillary density and fibrosis ($p < 0.05$ was considered significant).

3. Results 3.1. Cardiomyocyte Hypertrophy

There was a progressive increase in cardiomyocyte diameter with age: 12.2 ± 0.8 μm (juvenile), 16.4 ± 1.2 μm (adult), and 22.1 ± 1.6 μm (aged) ($p < 0.01$). H&E staining revealed enlarged nuclei, cytoplasmic granularity, and occasional vacuoles in aged myocytes.

3.2. Fibrotic Remodeling

Masson's Trichrome staining demonstrated a significant increase in fibrotic area: 4.8% (juvenile), 9.1% (adult), and 18.7% (aged) ($p < 0.001$). Fibrosis was predominantly perivascular and interstitial. Correlation analysis showed a strong positive correlation between age and fibrosis ($r = 0.91$).

3.3. Capillary Density and Vascular Remodeling

CD31 staining showed reduced capillary density with aging: 1480 ± 110 cap/mm² (juvenile), 1205 ± 95 cap/mm² (adult), and 885 ± 75 cap/mm² (aged). Capillary-to-myocyte ratio significantly decreased in aged myocardium ($p < 0.001$), indicating impaired angiogenesis.

3.4. Structural Disorganization

Aged myocardial sections exhibited increased intercellular space, myofibrillar disarray, nuclear pleomorphism, and subcellular damage. These degenerative features were absent or minimal in juvenile hearts, supporting cumulative structural compromise with age.

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4. Discussion

Our study confirms that cardiac aging is associated with pronounced structural remodeling affecting both muscle fibers and capillary networks. Cardiomyocyte hypertrophy likely reflects compensatory responses to increased wall stress and decreased contractile reserve (Dai et al., 2012). However, such hypertrophy becomes maladaptive when accompanied by mitochondrial dysfunction, loss of contractile proteins, and limited angiogenesis. Fibrosis contributes significantly to myocardial stiffening and loss of compliance. The upregulation of fibrogenic cytokines such as TGF-β and connective tissue growth factor (CTGF) promotes fibroblast activation and collagen synthesis. The increased fibrotic burden in aged myocardium, observed in our study, mirrors findings from both animal models and aged human hearts (Lakatta & Levy, 2003).Capillary rarefaction compromises nutrient and oxygen delivery, particularly detrimental in hypertrophied myocytes with elevated metabolic demands. Studies have shown that angiogenic signaling (e.g., VEGF) declines with age, contributing to impaired microvascular regeneration (Tomanek, 2005). The decreased capillary-to-myocyte ratio in our aged group underscores the mismatch between supply and demand.Moreover, the structural disarray, including myofibrillar disruption and nuclear abnormalities, suggests oxidative damage and compromised proteostasis. These features are hallmarks of advanced cardiac aging and have been linked to cellular senescence and impaired autophagy mechanisms.

5. Conclusion

This comparative histological study reveals that aging induces significant morphological changes in cardiac muscle fibers and capillary networks. The aged myocardium is characterized by myocyte hypertrophy, interstitial fibrosis, reduced capillary density, and structural disorganization. These changes collectively impair cardiac function and elevate the risk for cardiovascular disease in the elderly. Our findings emphasize the importance of targeting fibrosis, promoting angiogenesis, and preserving myocardial structure as therapeutic strategies in cardiac aging research.

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