Receptor identification in canine valvular interstitial cell culture

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P.12

IN VITRO TECHNIQUES TO STUDY VALVULAR INTERSTITIAL CELLS OF MITRAL VALVE IN DOMESTIC ANIMALS. A CELLULAR APPROCH FOR THE INVESTIGATION OF VALVULAR INSUFFICIENCY.

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Myxomatous mitral valve degeneration (MMVD) is the most common acquired cardiac disease in older dogs and it results in predominantly mitral valve regurgitation of the mitral valve and subsequent left-sided volume overload and eventually congestive heart failure. Despite many clinical investigations several questions still remain concerning etiopatholgical mechanisms. Valvular interstitial cells (VICs) are the most prevalent cell type in cardiac valves but t they have not been extensively studied in the dog. The study of cellular and molecular mechanisms of valvular interstitial cells (VICs) is an emerging research area for veterinary medicine and comparative pathology. Authors decided to validate a reliable VICs cultivation technique using bovine mitral valves collected at slaughter house, and conserved in cold and sterile phosphate buffer saline (PBS). In lab, atrial aspect of the mitral leaflets was removed scraping with a scalpel: subendocardial material was collected and seeded with complete medium (DMEM, FBS, antibiotic and antimycotic solution, L-glutamine), and routinely incubated. This minced material was seeded on glasses: at 80% confluence, they were rinsed with 4% formalin (10 min) and then treated for immunocitochemistry (ICC) using vimentin, factor VIII, and actin smooth muscle antibodies. The ICC showed a strong and diffuse cytoplasmic positivity for vimentin and occasional for actin smooth muscle. All cells were negative for factor VIII, thus excluding the endothelial origin. These results demonstrated that this isolation method is able to primary isolate VICs from bovine mitral valve. The data obtained by this preliminary assay demonstrated that it is possible to isolate and start a primary culture from bovine mitral valve leaflet, and that ICC was able to confirm that only VIC cells were present in the culture. Authors performed the same procedure also with mitral valves collected by dogs, and these cells have been investigated to understand better the receptor network during mitral valve disease (results presented elsewhere).

P.13

RECEPTOR IDENTIFICATION IN CANINE VALVULAR INTERSTITIAL CELL CULTURE

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Myxomatous degeneration occurs when the valve becomes thickened with formation of small nodules on the edges of the leaflets, avoiding complete closing and blood can flow backward into the left atrium. Severe remodeling of the spongiosa and fibrosa layers is due to proliferation of myxomatous tissue in which valvular interstitial cells (VICs) play an important role. The study of cellular and molecular mechanisms of VICs is an emerging research area in human and veterinary medicine, because this pathology could arise spontaneously in animal species (i.e.: dogs). According to the method previously validated, VICs from an healthy dog were collected and cultivated. After routinely cultivation steps, cells were seeded directly on glasses in Petri dishes. At 50% of confluence, cells were fixed with 4% buffered formalin and conserved with phosphate buffered saline (PBS) and 0.03% of sodium azide. ICC was performed with Transient receptor potential vanilloid 1 (TRPV1, dilution 1:50), adenosine (AD)1 (dilution 1:50), Tumor growth factor (TgF)ß1 (diluition 1:100), and Ki-67 antibodies (Santa Cruz Biotechnology). To confirm the only presence of VICs, incubation with vimentin, factor VIII, and actin smooth muscle antibodies was performed. Positive and negative controls were always maintained. Canine VICs were positive for all antibodies: 1) TRPV1 is expressed in both physiological and pathological conditions. Its identification was previously established in humans and dogs, but never in VICs cells. Its role is not clear yet but it might be involved in the mixomatous degeneration. 2) It is known that TgFß is able to induce differentiation of valvular endotelial cells (VEC) in VICs in humans. The identification of this receptor in this cells and a over expression or activation could lead to floppy valve or prolapsed of mitral valve. 3) AD1 receptor activation could exert antihypertrophic effect on the heart after mitral valve insufficiency. 4) Ki-67 strain confirmed that cells have an high mitotic turn over. In the present case, all these receptors have been identified in physiological condition, collecting cells from an healthy canine mitral valve. Further study will be necessary to characterize receptors and to understand their role in pathological conditions (i.e.: cell culture in presence of oxidative stimuli mimicking premature senescence). Moreover several physiological and pathological cases must be enrolled, and ICC must be performed. The final aim is to understand if a medical therapy could be attempt in order delay the need for surgical intervention or patient's death.