

## Distribution of costameric proteins in normal human ventricular and atrial cardiac muscle.

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**Abstract:** In the mature heart, the intercalated disc and costameres provide the cell-cell and cell-matrix junctions respectively. Intercalated disc is situated at the bipolar ends of the cardiomyocytes and the myofibrils are anchored at this structure. The costameres mediate integration with the extracellular matrix that covers individual cardiomyocytes laterally. Costameres are considered as "proteic machinery" that appears to comprise two protein complexes: the dystrophin-glycoprotein complex (DGC) and the vinculin-talin-integrin system. There are structural differences between atrial and ventricular myocytes, but there have been relatively few studies that have analyzed costameres and focal adhesion function in cardiac cells. Our previous study carried out only on atrial myocytes, demonstrated that the DGC and talin-vinculin-integrin complexes had a costameric distribution that, unlike skeletal muscle, it localized only on the I band. We performed a further immunohistochemical analysis extending also the evaluation to the normal human cardiac muscle fibers obtained from ventricle and interventricular septum, in order to define the distribution and the spatial relationship between the proteins of the two complexes also in the other heart districts. Immunofocal microscopy of cardiac tissue revealed the costameric distribution of DGC and of vinculin-talin-integrin system, the association of all tested proteins in intercalated disks, in disagreement with other Authors, and in T-tubule with irregular spokelike extensions penetrating toward the center of the cell. Moreover, our data showed that all tested proteins colocalize between each other.

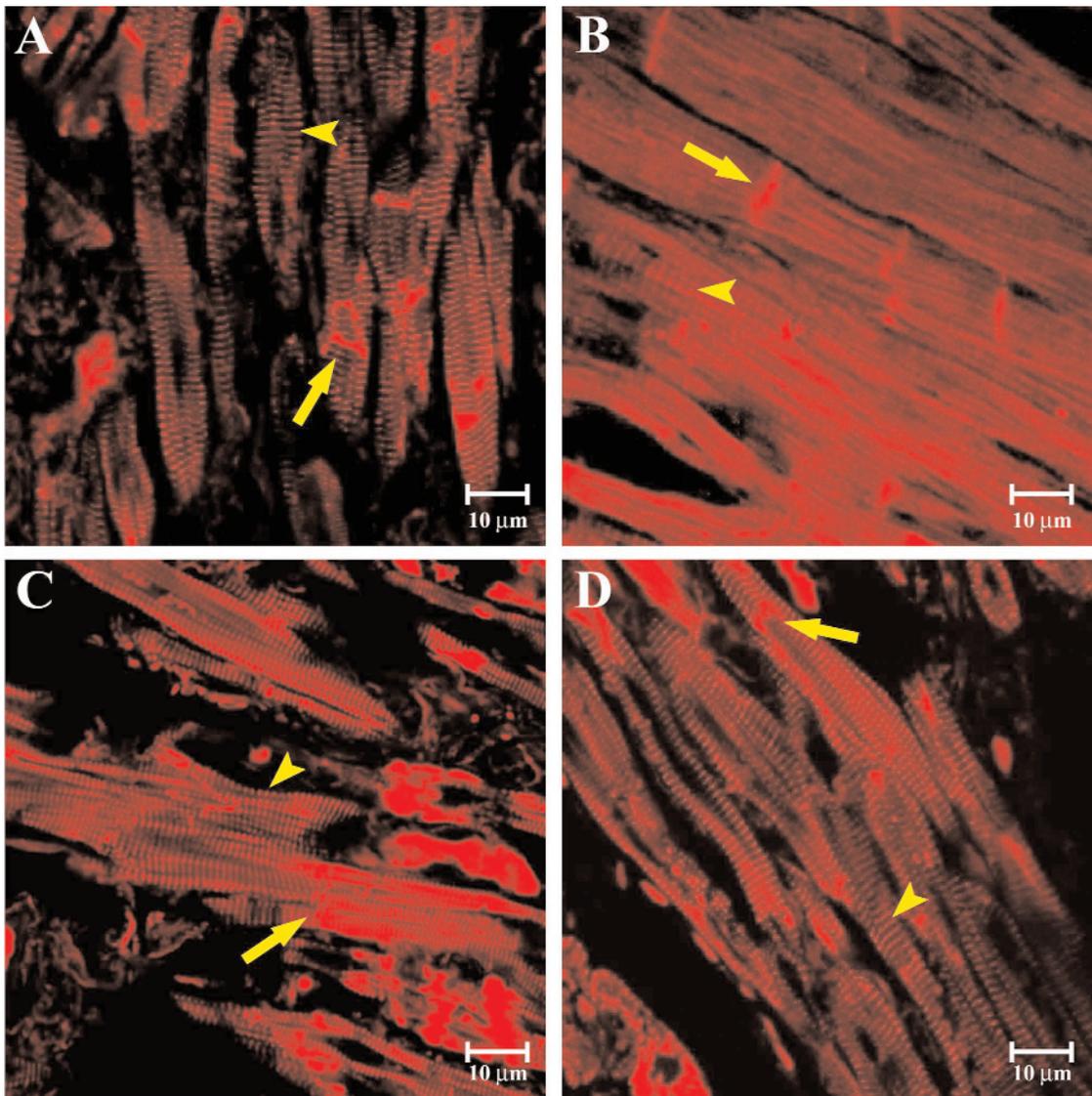
**Key words:** atrium, ventricle, costameres, intercalated disk, T-tubules, immunohistochemistry,

### Introduction

Cardiac myocytes are under continuous mechanical stress and the signal transduction pathways that are activated in response to mechanical forces include many unique components. Cell-cell and cell-matrix junctions in cardiac myocytes are termed intercalated discs (ICDs) and costameres, respectively. The ICDs support synchronized contraction of cardiac tissue and provide a electro-mechanical coupling [1]. The grid-like structure of costameres, which encircle the myocyte perpendicular to its long axis, participates in signaling functions and reinforces mechanically the sarcolemma, thereby helping to protect muscle cells against workload-induced membrane damage [2-3]. Costameres appear to comprise two protein complexes: the dystrophin-glycoprotein complex (DGC) and

the vinculin-talin-integrin system [4-5]. There have been relatively few and discordant studies that have analyzed costameres in cardiac cells with the well note structural differences between atrial and ventricular myocytes. In particular, about dystrophin, some Authors showed that it is not uniquely distributed at costameres and is continuously and uniformly distributed at the cytoplasmic surface of the peripheral plasma membrane [6], while Kaprielian *et al.* [7] have documented that dystrophin partially colocalizes with costameric vinculin in myocytes and it is absent from ICDs. Therefore, dystrophin also localizes at the T-tubules in cardiac muscle in contrast to its known absence in skeletal muscle T-tubules [8]. Our previous study carried out only on atrial myocytes, demonstrated that the DGC and talin-vinculin-integrin complexes had a costameric distribution and both complexes are present at the level of T tubules and ICDs [9]. The aim of this study is a further immunohistochemical analysis on normal human cardiac muscle fibers obtained from ventricle and interventricular septum in order to define

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**Fig. 1.** Cryosections labelled with antibodies to  $\alpha$ -sarcoglycan (A),  $\beta$ -dystroglycan (B), dystrophin (C) and talin (D) showing the labelling at ICDs (arrows) and costameres (arrowed).

the localization and the relationship between the proteins of the two complexes at the level of ICDs, costameres and T Tubules also in the other heart districts.

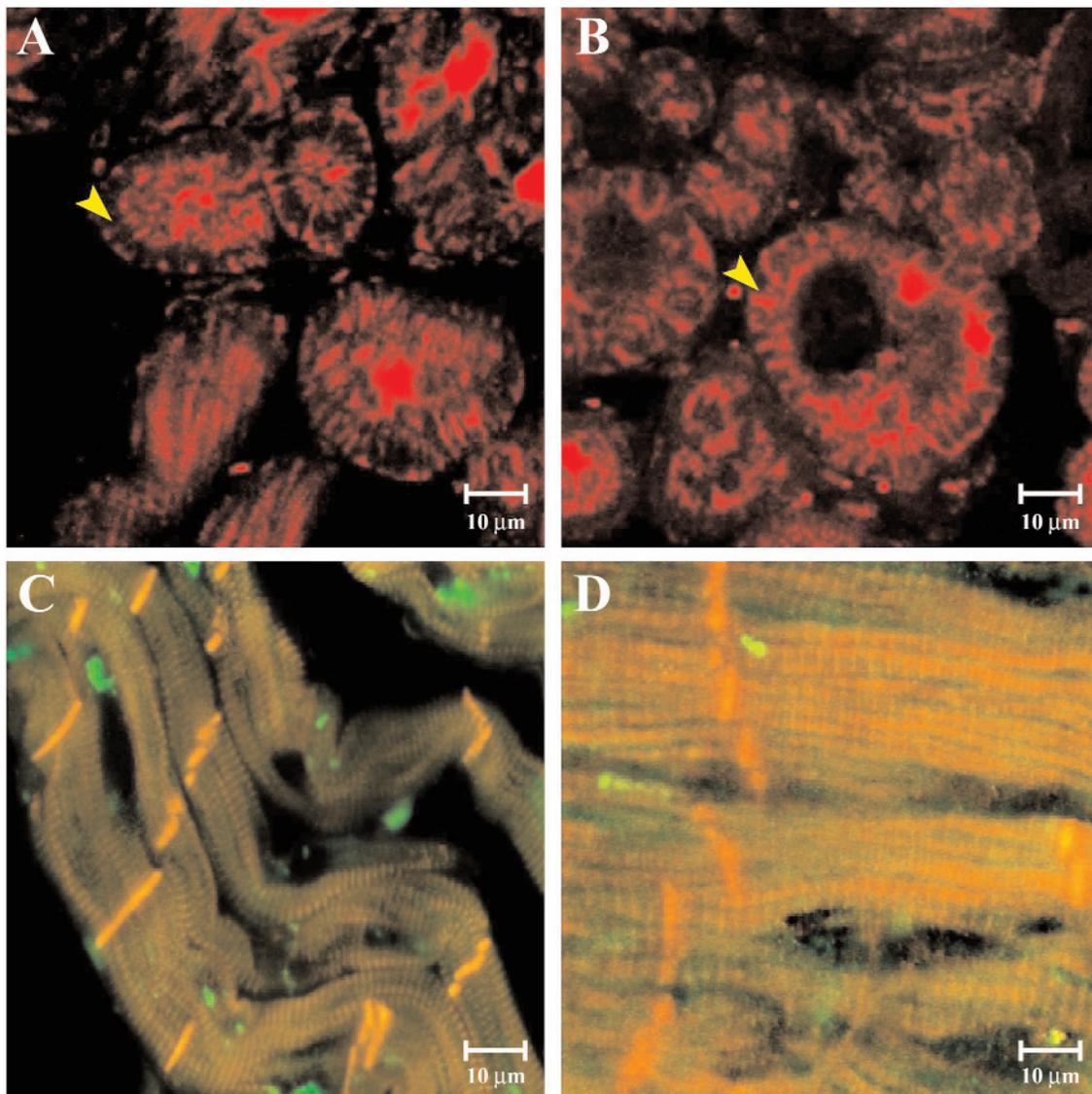
### Materials and methods

Normal human biopsies of left ventricle and interventricular septum were obtained from patients who underwent surgery after informed consent. They were fixed in 3% paraformaldehyde in a 0.2 M phosphate buffer at pH 7.4, infiltrated with saccharose at 12% and at 18%, and frozen in liquid nitrogen. Ten-mm-thick cryosections were permeabilized with 0.1% TRITON X-100 in PBS for 10 mins. Samples were pre-incubated for 1hr. in blocking solution (PBS plus 1% bovine serum albumin-BSA and 10% goat serum-GS). In order to define a model of distribution of DGC and vinculin-talin-integrin system proteins, we analyzed all proteins with both single and double immunolabeling techniques on longitudinal and transverse sections, with different series of single and double localizations. Antibodies used were:  $\alpha$ -,  $\beta$ -,  $\delta$ -,  $\gamma$ - and

$\epsilon$ -sarcoglycan, diluted 1:100, 1:200, 1:50, 1:100, and 1:100 respectively (Novocastra Laboratories Ltd),  $\alpha$ -, and  $\beta$ -dystroglycan, both diluted 1:100, and dystrophin diluted 1:20 (Novocastra Laboratories Ltd), vinculin and talin both diluted 1:100 (Sigma-Aldrich). In all single immunolabellings, TRITC-conjugated IgG anti-mouse in goat (1:100; Jackson ImmunoResearch Laboratories) was used; for double-localization reactions, sections were incubated with a second antibody conjugated with FITC conjugated secondary IgG, as second fluorochrome (1:100 dilution; Jackson ImmunoResearch Laboratories). Sections were then observed and photographed using a Zeiss LSM 5 META laser scanning microscope (Carl Zeiss).

### Results and discussion

The results of present study showed that all tested proteins had costameric distribution and a constant localization in T-tubule and ICDs too. Moreover, double-immunofluorescence showed that all tested proteins of the



**Fig. 2.** Cryosections labelled with antibodies to vinculin (A) and  $\alpha$ -dystroglycan (B). Merged immunofluorescence images of cryosections labelled with  $\epsilon$ -sarcoglycan (red)/ $\beta$ -dystroglycan (green) (C) and talin (red)/ $\alpha$ -sarcoglycan (green) (D).

two systems, colocalized with each other. Single immunolabelling on longitudinal sections of all tested proteins (Fig. 1), evidenced their presence in ICDs (arrows) and costameres (arrowed). In transverse sections, all tested proteins showed irregular spokelike extensions of the myocyte sarcolemma, penetrating toward the center of the cell that indicated T-tubules (arrowed). In the double-localization images, a clear yellow fluorescence revealed the colocalization of all tested proteins in all different myocardial districts.

The concentration of these proteins over the costameric regions of the human cardiac fibers of ventricle and interventricular septum, coincides with that previously observed in the atria, making to hypothesize that there are no functional differences among the

present proteins in the atria and those of the ventricle or septum. It is evident the presence of tested proteins additional junctional domains in comparison to the costameres, even if remains still to appraise their role in intercalated disks or T tubules. It is possible to hypothesize that in the ICDs, they can provide to a structural and functional integration of the myofibrils together to the junctional systems of the same disk. Regarding T-tubules our results also showed the presence of all tested proteins implying an additional role the proteins in the E-C coupling.

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