ANNEXIN A2 REGULATES APICAL JUNCTIONAL COMPLEX ORGANIZATION IN COLORECTAL CANCER CELL LINES

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INTRODUCTION

❖ Annexin A2 (ANXA2) is a calcium-dependent phospholipid binding protein responsible for multiple functions in the cell[1]. Studies suggest ANXA2 as a component of the apical junctional complex (AJC), considering that ANXA2 suppression inhibits E-cadherin reestablishment in cellular contacts damaging cell-cell adhesion and it's colocalization with tight junction proteins[2,3].

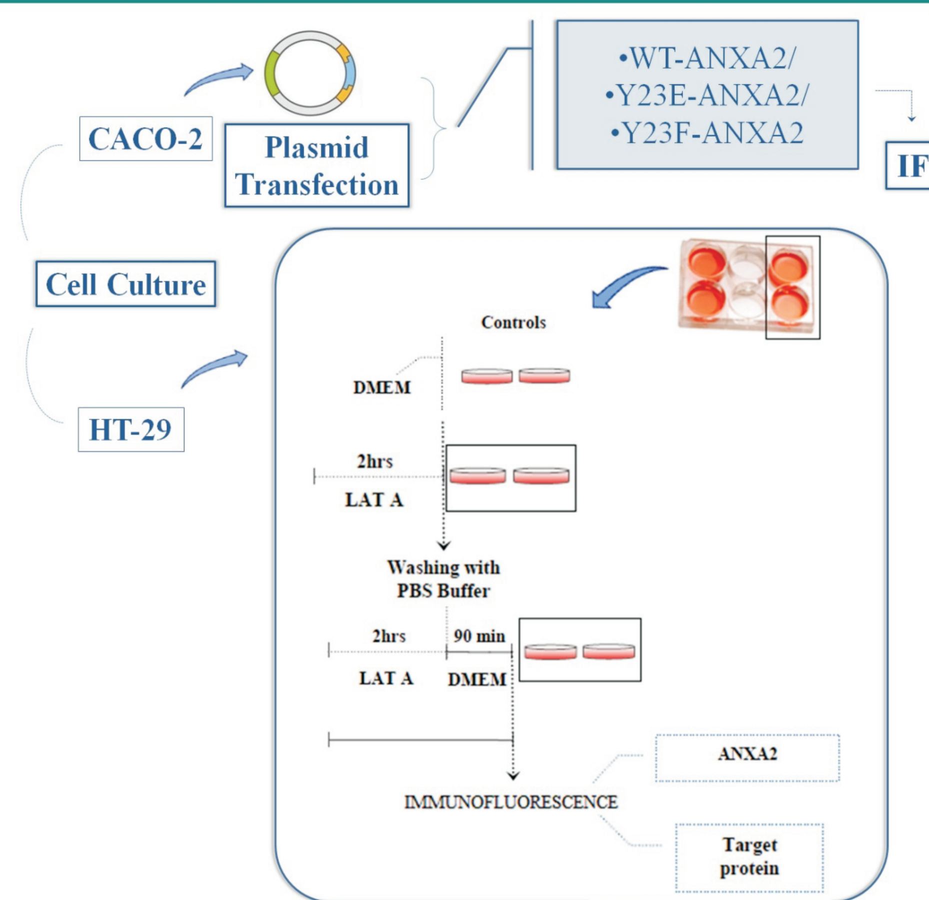
❖ AJC disorganization is associated with carcinogenesis, resulting in morphological changes due to cellular contact loss (critical step of the metastatic cascade)[4,5].

❖ Colorectal cancer (CRC) ranks fourth as deadliest cancer in the world, being third most prevalent among men and second among Brazilian women[6]. ANXA2 is overexpressed in distinct tumor types (CRC among them) and might be associated with the epithelial mesenchymal transition (EMT), a process that requires AJC rearrangement[7,8,9].

OBJECTIVE

Analyze the influence of Annexin A2 on the formation, maintenance and disorganization of the apical junctional complex in the HT-29 and Caco-2 colorectal cancer cell lines.

METHODS



RESULTS

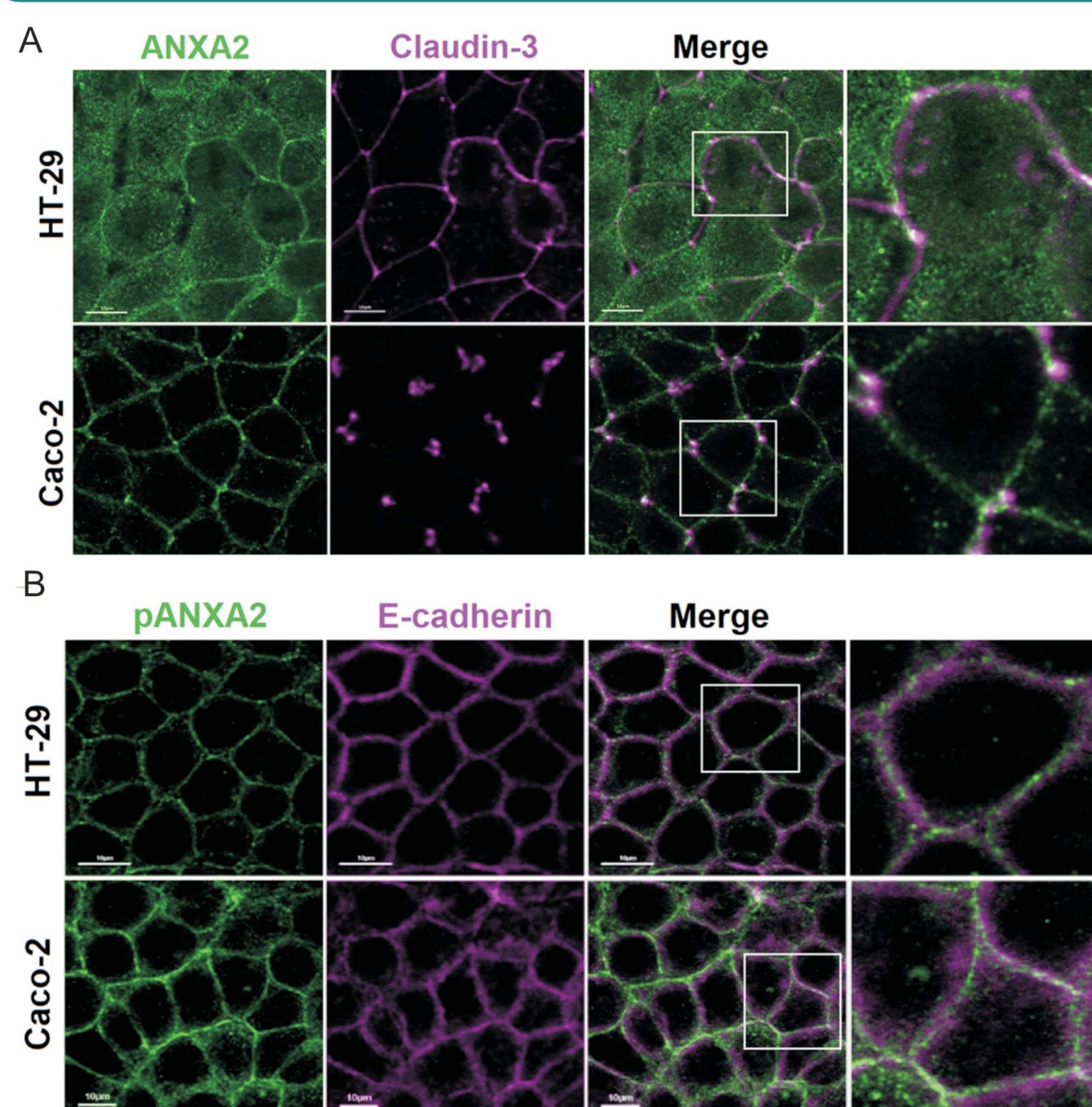


Fig.1- ANXA2 and apical junctional complex proteins in the HT-29 and Caco-2 colorectal cancer cell lines. Representative immunofluorescence images by confocal microscopy of ANXA2 and claudin-3 **(A)**; pANXA2 and E-cadherin proteins **(B)**. The merge indicates the co-localization between ANXA2 (or) pANXA2 and these other target proteins. An enlargement of the areas defined by the white boxes are shown on the right of the merged images. Scale bars=10 µm.

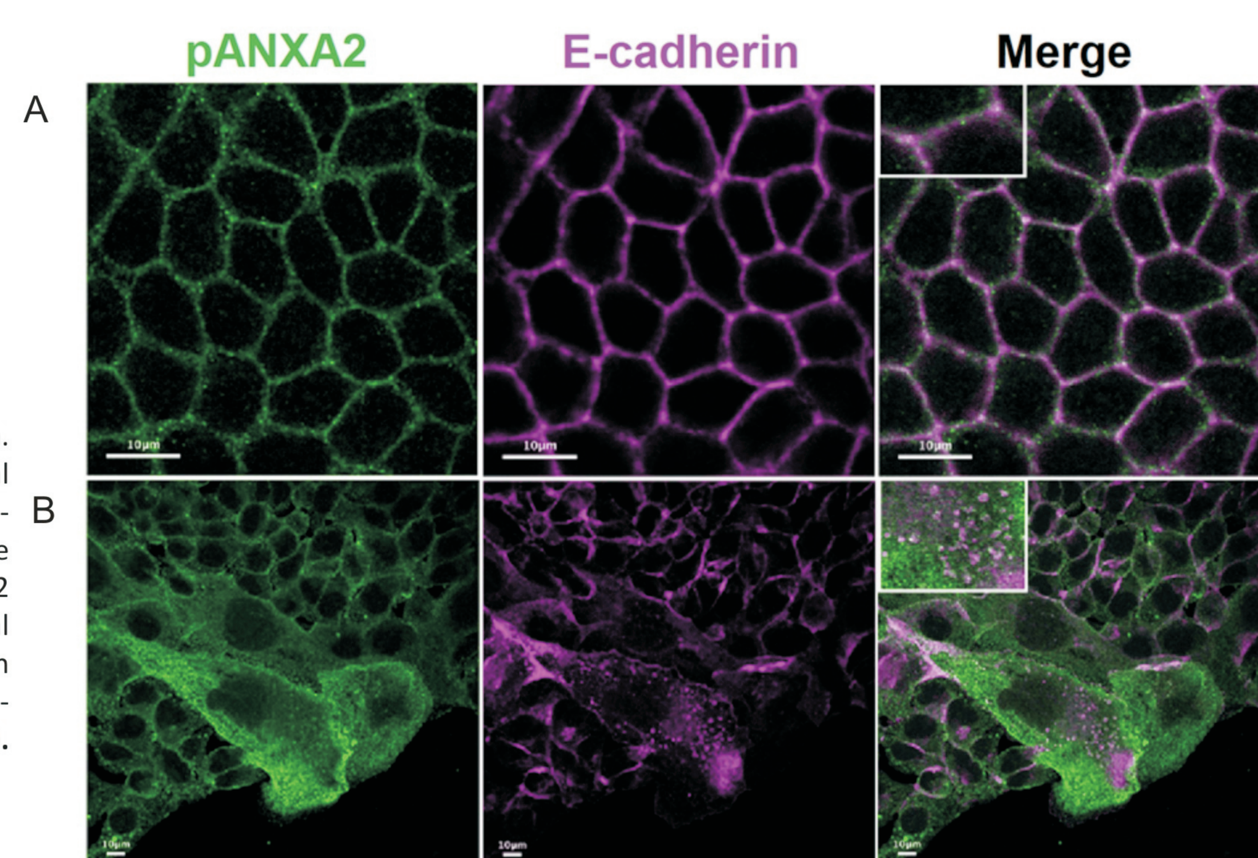


Fig.2- ANXA2 on tumor cell migration. Immunofluorescence images by confocal microscopy analysis of pANXA2 and E-cadherin proteins in HT-29 cell line. The merge shows co-localization of pANXA2 and E-cadherin in an organized epithelial tissue **(A)** and in migratory cells with superexpression of pANXA2 and E-cadherin spots all over the cytoplasm **(B)**. Scale bars=10 µm.

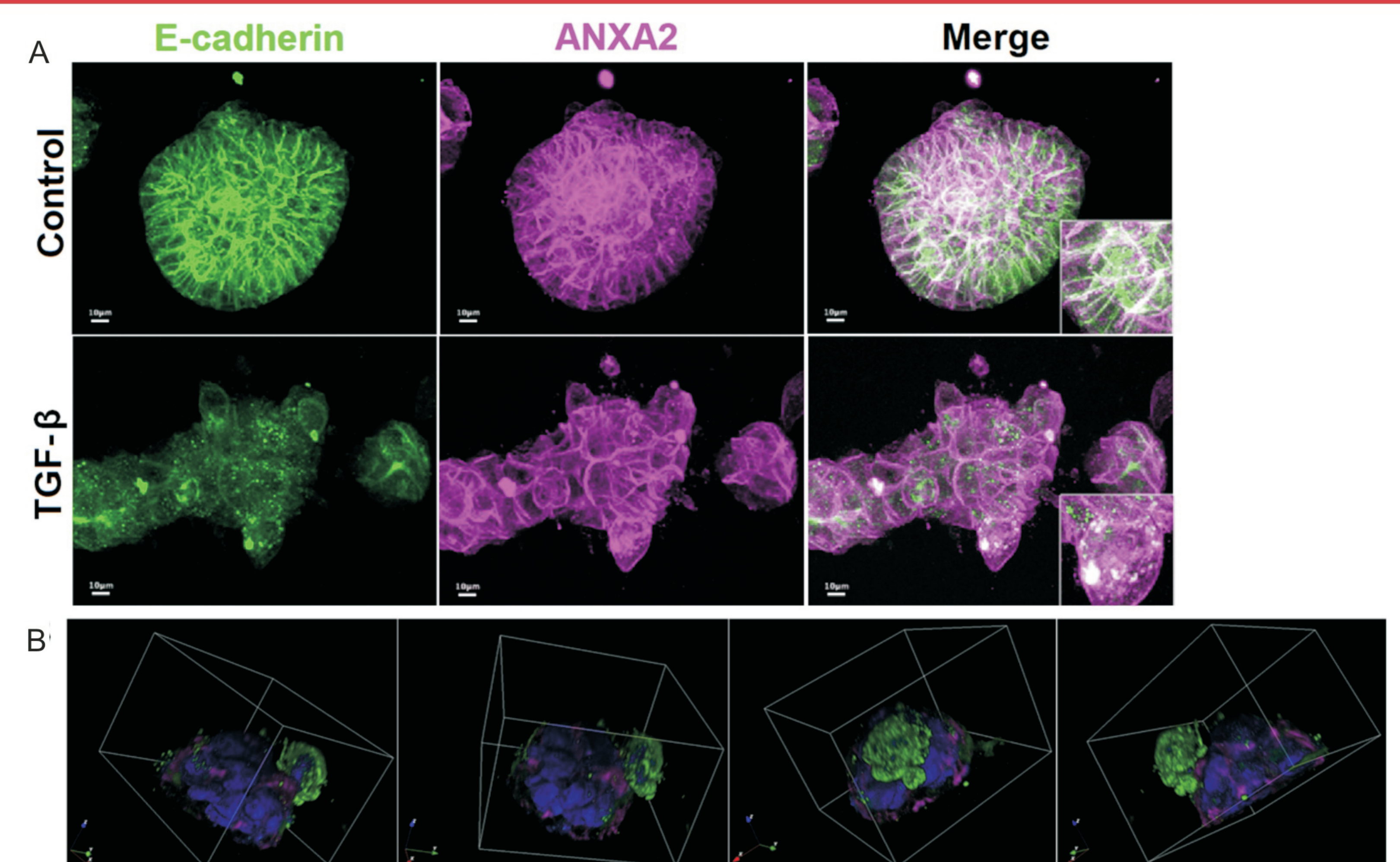


Fig.3- EMT induction alters ANXA2 and E-cadherin organization. Immunofluorescence of HT-29 spheroids show co-localization between ANXA2 and E-cadherin at the control and a disorganization on this 3D culture cell upon TGF-β treatment **(A)**. Scale bars=10 µm. Caco- 2 spheroids exhibit migratory cells superexpressing ANXA2 upon TGF-β treatment ; nuclei were stained with DAPI **(B)**.

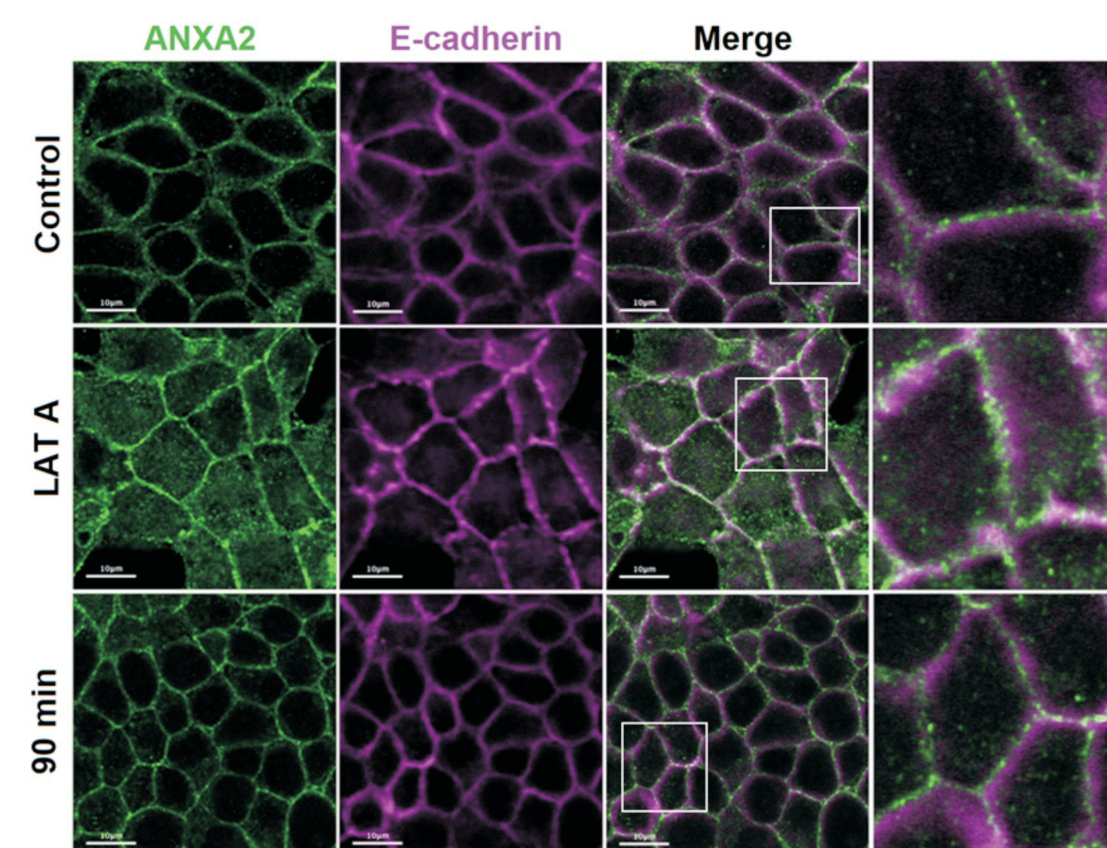


Fig.4- Deregulation of the apical junctional complex. Images analyzed by confocal microscopy of HT-29 cell line with ANXA2 and E-cadherin staining after Latrunculin A treatment and after washing followed by a 90 minutes recovery time to the proteins reestablishment on the AJC. The merge indicates the co-localization between ANXA2 and E-cadherin. An enlargement of the areas defined by the white boxes are shown on the right of the merged images. Scale bars=10 µm.

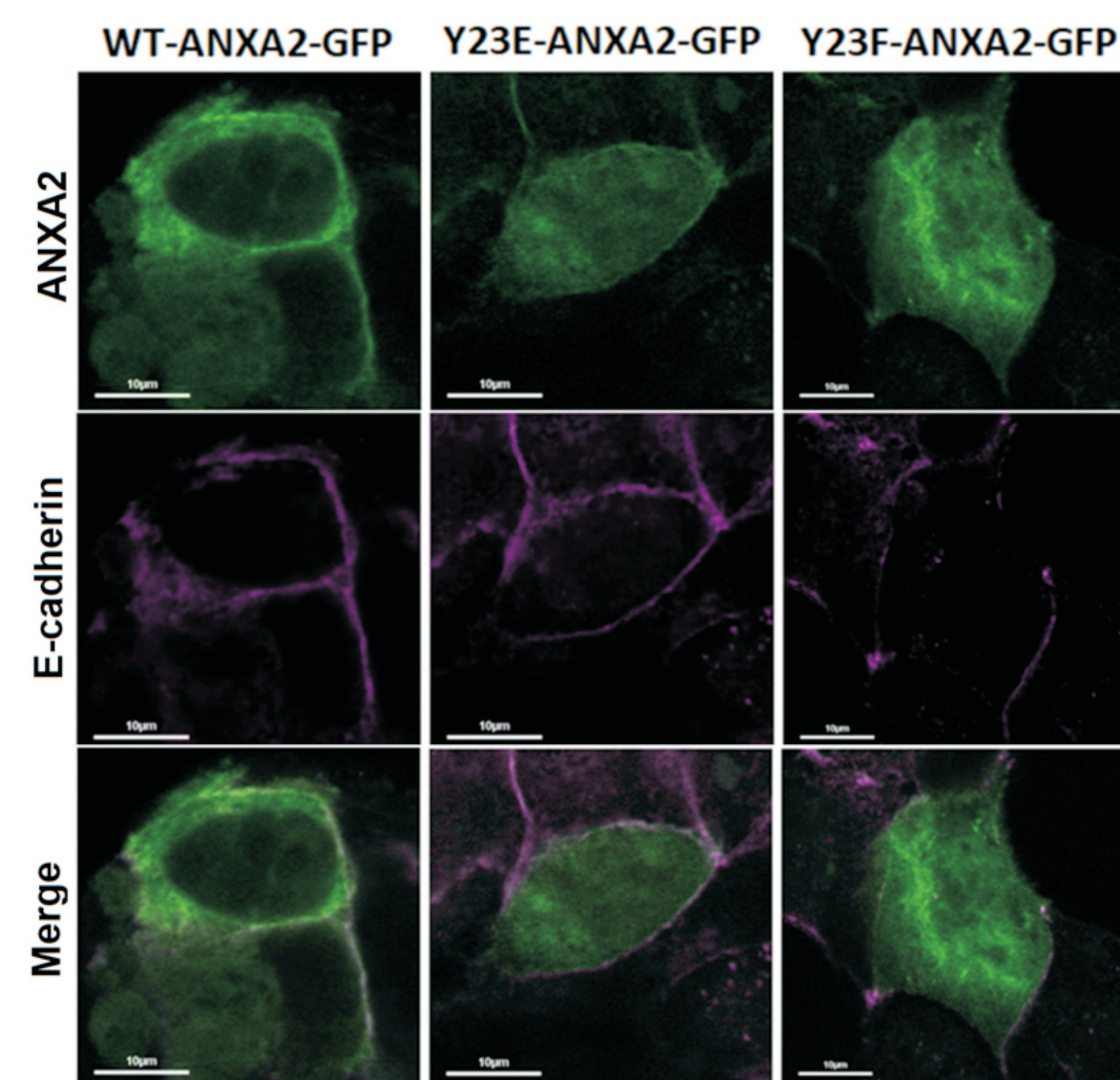


Fig.5- Interaction between ANXA2 mutant isoforms with the E-cadherin protein. Immunofluorescence images analyzed by confocal microscopy of Caco-2 cell line superexpressing ANXA2 wild type (WT-ANXA2-GFP); fosfo-mimic (Y23E-ANXA2-GFP) and fosfo-inhibitor (Y23F-ANXA2-GFP) isoforms conjugated with green fluorescent protein (GFP) by plasmid transfection. E-cadherin staining with Alexa-546. The merge indicates the co-localization between ANXA2 mutant isoforms and E-cadherin. Scale bars=10 µm.

CONCLUSION

These results have shown that ANXA2 is indeed essential for adherens junction formation and consequently AJC structuration. Taking this into account and all data from the literature we suggest ANXA2 as a possible target in cancer therapy focused on the prevention of the metastatic cascade.

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