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MDPI

NON-GENOMIC ACTION OF ANDROGEN RECEPTOR IN COLORECTAL CANCER

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INTRODUCTION & AIM

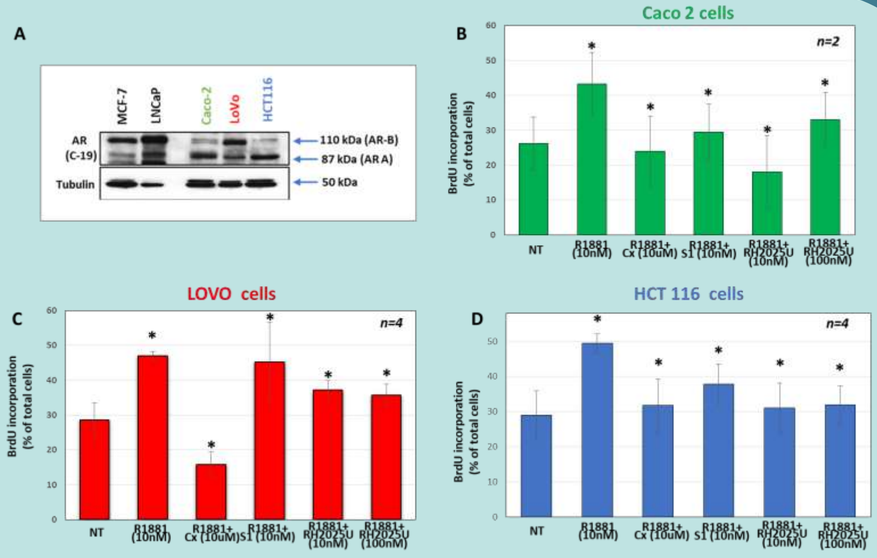
Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related deaths worldwide. Epidemiological studies demonstrate that men have a higher CRC incidence rate and face worse survival outcomes compared to women. This gender disparity in CRC incidence and outcomes pushed us to explore the mechanisms by which the androgen receptor (AR) may influence its pathology. Interestingly, it is known that the roles of AR in cancer extend beyond traditional hormone-dependent cancers. Understanding how AR mediates CRC aggressiveness could provide valuable insights that may help find more effective treatment strategies.

METHOD

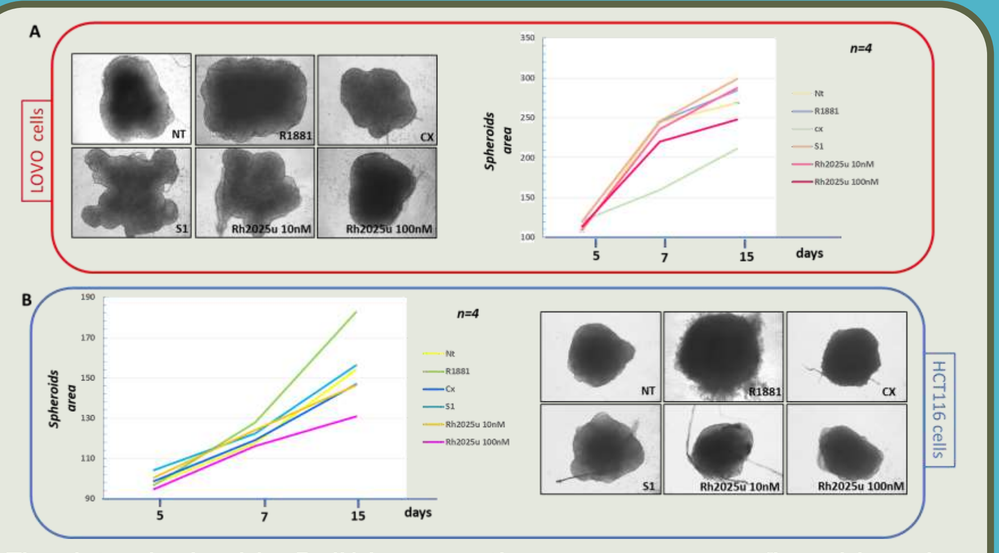
In this study, we used CRC-derived Caco2, LoVo, and HCT-116 cells, expressing AR at different extend. BrdU incorporation assay and the measurement of spheroid growth were used to follow cell proliferation. Biochemical approaches such as co-immunoprecipitation and immunoblot show that the androgen treatment induces the association between AR and Filamin A and the activation of different effectors that control androgen-dependent CRC cells proliferation.

RESULTS & DISCUSSION

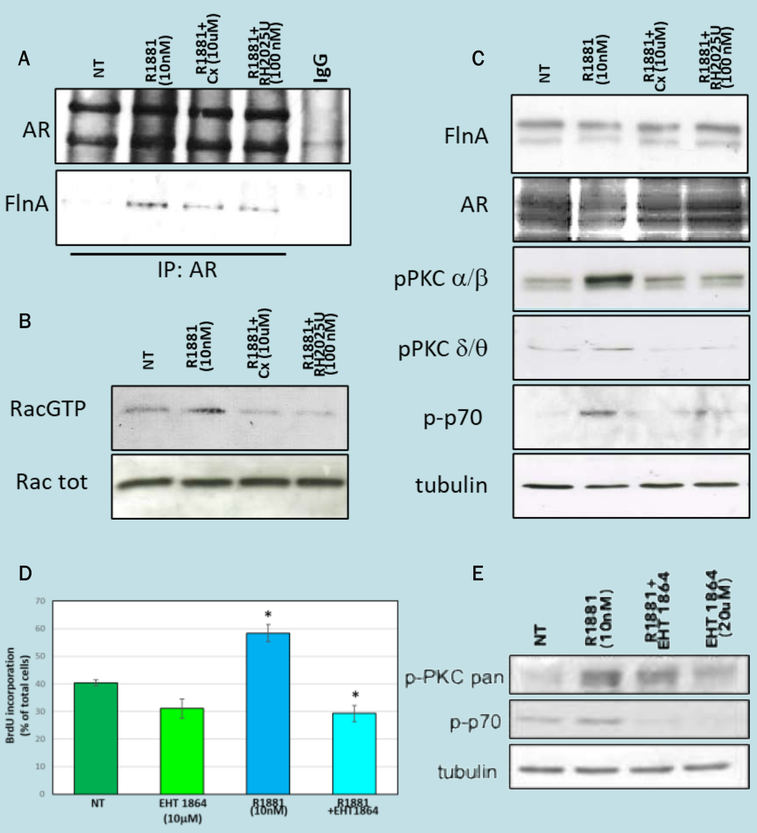
Three CRC-derived cell lines (Caco,-2, LoVo, and HCT116), representing different stages of colon cancer, were used. All these cells express both isoforms of the androgen receptor AR, although in different amount (A). The 10nM R1881 treatment increases the BrdU incorporation in colon cancer cells (B, C and D). The use of the antiandrogen Bicalutamide (Casodex 10mM) suggests that, in these models, cell proliferation is controlled by AR.



In this analysis, two small peptides S1 and Rh2025u, designed to mimic the AR sequences responsible for the interaction of the receptor with c-Src or Filamin A, respectively, were also used. In particular the RH2025U peptide reverse the androgen-induced effect more efficiently than S1 peptide. These results indicate not only that AR controls cell proliferation in CRC cells, but also that it works by interacting with FlnA.



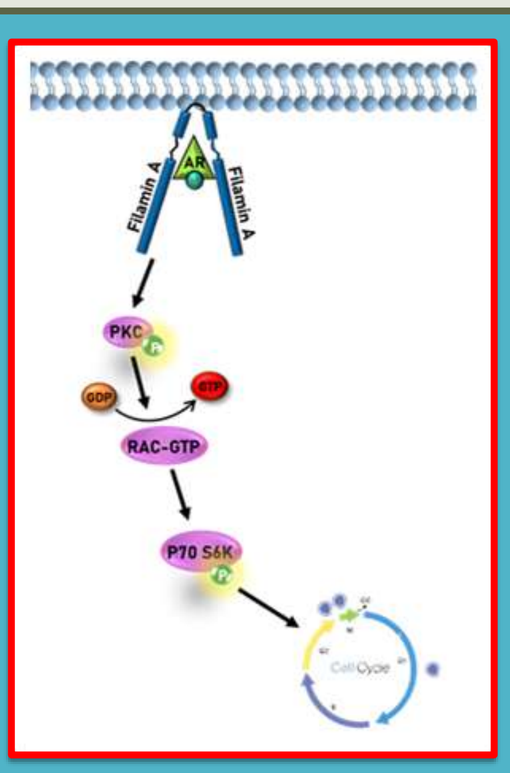
The data obtained by BrdU incorporation assays were confirmed by using 3D models. LoVo (A) and HCT116 (B) cells were used for spheroids formation in ultra-low attachment round bottom 96 well plates. Five days after seeding cells, the spheroids were treated every two days with R1881 in the absence or presence of the antiandrogen Casodex or the peptide S1 at 10nM, or the peptide Rh2025u at two different concentrations (10 and 100nM). The spheroid size was calculated by using the ImageJ software and the results were reported in the graphs (A and B upper right and lower left panels). The data shows that the growth of LoVo spheroids is strongly reduced by both Casodex and the Rh2025u at 100nM concentration, while the HCT116 spheroids' size increase was strongly reduced by the 100nM Rh2025u treatment.



Considering that Rh2025u was the best inhibitor both in LoVo and in HCT116 cells in particular, we analyzed the molecular pathway controlled by the AR/FlnA complex, responsible for the colon cancer cell proliferation. We performed a Co-IP assay (A) in HCT116 serum-starved cells treated as indicated in Fig.A and tested that the R1881 treatment induced the AR/FlnA complex formation. The complex was disrupted both by Casodex and by Rh2025u treatment. Furthermore, in serum-starved HCT116 cells, by performing a Rac activation assay (B) and an analysis of cell lysates (C), we observed that the R1881 treatment activated different effectors such as PKC, Rac and p70.

To partially check if these effectors were involved in cell proliferation, we performed a BrdU incorporation assay (D) in HCT116 cells untreated or treated with R1881 in the presence or absence of the Rac inhibitor, EHT1864. The results confirmed that Rac participates in controlling androgen-dependent cell proliferation in colon cancer cells. Furthermore, through cell lysate analysis (E), we also demonstrate Rac involvement in p70 kinase activation, differently from PKC, whose phosphorylation is dependent by AR/FlnA interaction.

Our findings demonstrate that AR specifically interacts with FlnA in androgen-treated CRC cells. These associations is responsible for the activation of different effectors such as Rac, PKC, and other proteins thereby controlling the proliferation of different CRC-derived cells.



CONCLUSION

The present study emphasizes the role of AR in CRC and highlights how this receptor could contribute to CRC onset and progression, especially in patients with altered concentrations of androgens. Clarifying the role of this steroid receptor in colon cancer can pave the way to developing new screening campaigns and new specific and effective therapies such as the use of the antiandrogens or AR-targeting peptides to cure this tumor.

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