The OSCC cells sensitivity to 5-AZA treatment is due to ANXA1 overexpression.

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INTRODUCTION

Squamous cell carcinomas of the oral cavity (OSCC) represent the most frequent forms of head and neck (HNSCC). OSCC is classically associated with a history of alcohol and tobacco abuse, but its etiological correlation with human papilloma virus (HPV) infection is well established, as well [1]. Despite recent advances in the investigation and treatment of this disease, including also adjuvant therapy, mortality remains high. Some strategies have been applied in patients to prevent chemotherapy resistance [2] including the treatment with 5-azacitidine (5-AZA).

5-AZA was associated, allowing us to hypothesize the demethylation of DNA, with an increase in transcriptional activity of tumor suppressor candidate genes. Currently, prognostic and predictive factors able to give indications about the biological aggressiveness of the OSCC are very few [3]. Thus it is crucial to find and study proteins that can be considered as reliable molecular biomarkers for HNSCC in order to be used for diagnostic, prognostic purposes and to discover new potential therapeutic targets.

Annexin-A1 (ANXA1) is a 37 kDa protein, belonging to the annexin family and capable of binding cell membrane phospholipids in a calcium-dependent manner. ANXA1 is known to participate in a number of pathophysiological mechanisms [4]. In HNSCC, it is now established that ANXA1 is downregulated, that it is involved in tumourigenesis and its lower expression is correlated to an advanced stage, confirming a close correlation between expression and staging [5].

AIM

This study aim to evaluate the effect of 5-AZA the aggressive behavior of OSCC cell lines by affecting the ANXA1 expression.

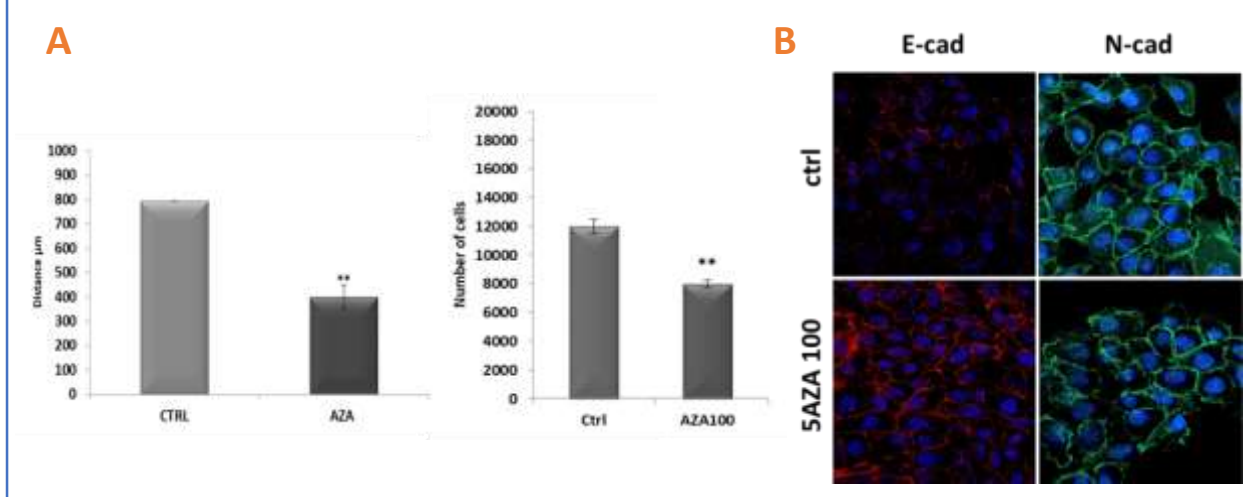
METHODS

HPV- (CAL27) and HPV+ (SCC154) OSCC cell lines were treated 24 hours with 5-AZA 100 µM (based on previous studies) and analyzed for the expression of ANXA1 by Western Blot (Fig. 1 A-C, Fig. 3 A) and immunofluorescence (Fig. 1 B, Fig. 2 B) . Next the drug effect on cellular aggressiveness was evaluated by motility assays (Fig. 2 A-B, Fig. 3 C) and by immunofluorescence of mesenchymal-epithelial transition (MET) markers, E-cadherin and N-cadherin (Fig. 2 B, Fig. 3 D).

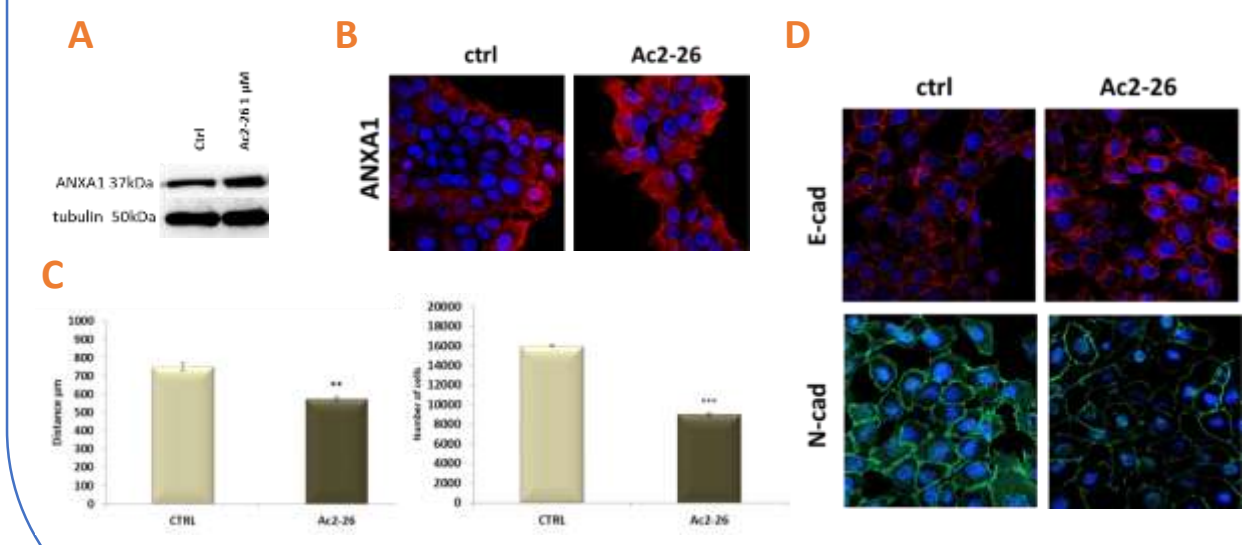
RESULTS

1 Treatment with 5-AZA (100uM) induces an increase in the expression of ANXA1 in CAL 27 (HPV-) cell line

2 The cell motility is reduced in CAL 27 cells with 5-AZA and the drug induces MET

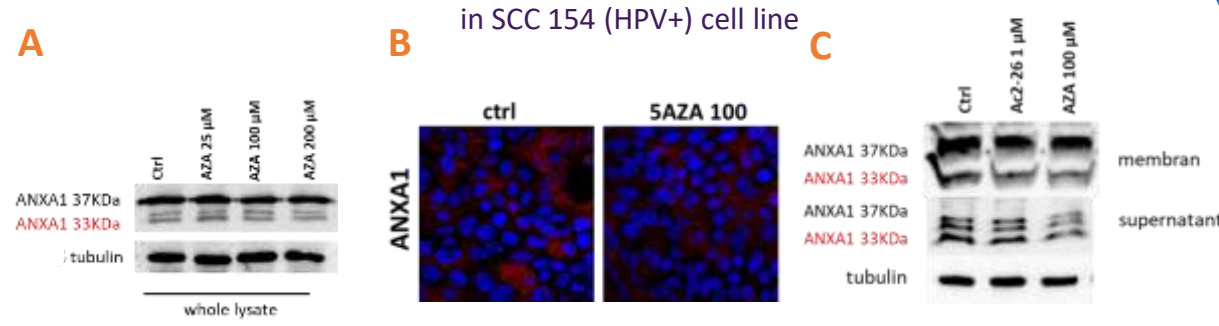


3 In the presence of treatment with Ac2-26(ANXA1 mimetic peptide) (1uM) cell motility in CAL 27 cells is reduced and the cells acquire a less aggressive phenotype (MET)

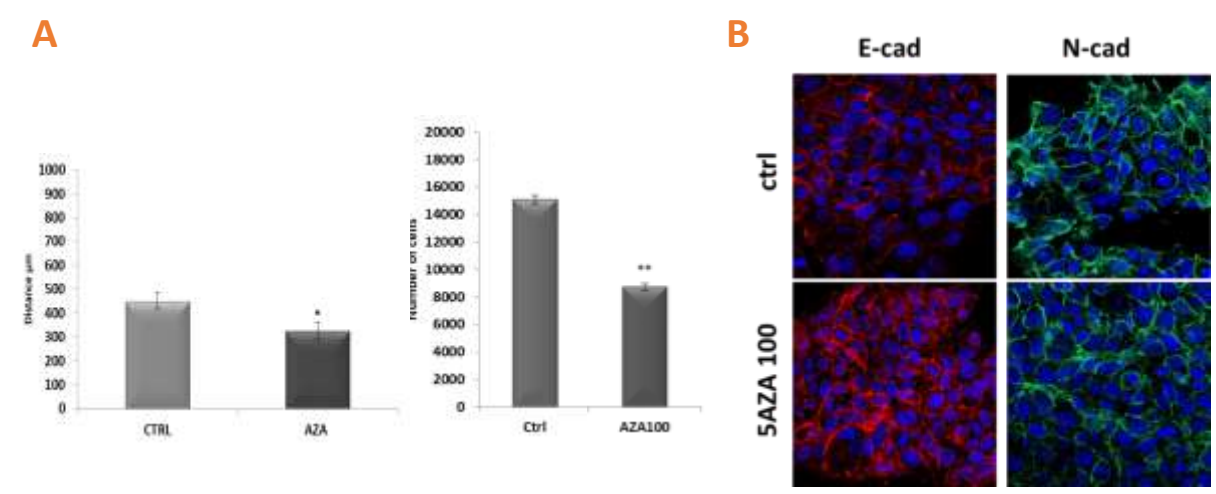


RESULTS

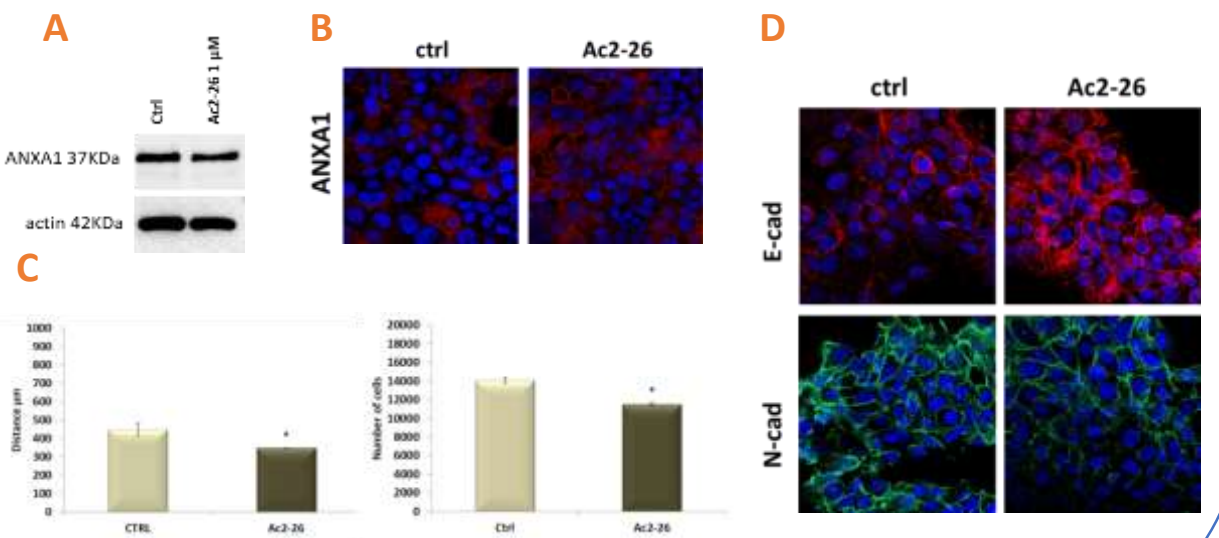
1 Treatment with 5AZA (100uM) does not induce an increase in ANXA1 expression in SCC 154 (HPV+) cell line



2 The cell motility is reduced in SCC1 54 cells with 5-AZA and the drug induces MET



3 In the presence of Ac2-26(ANXA1 mimetic peptide) treatment (1uM) cell motility in SCC 154 cells is reduced and cells acquire a less aggressive phenotype (MET)



CONCLUSIONS

- ✓ 5 AZA induces the acquisition of less aggressive phenotype in OSCC cell lines;
- ✓ In OSCC HPV- cell line this effect is ANXA1-mediated.

FUTURE PERSPECTIVES

- ➢ To broaden the data obtained on other cell lines (HPV- and HPV+);
- ➢ Confirm the involvement of ANXA1 modulating its expression by ANXA1 siRNAs;
- ➢ Study of the regulation (direct or indirect) of ANXA1 expression by 5 AZA.

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