Metformin and OSCC: reduction of the aggressive phenotype in a probable CAF-1 dependent manner

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the most aggressive subtypes of head and neck squamous cell carcinoma (HNSCC). Main risk factors are represented by tobacco consumption and heavy use of alcoholic, and to a lesser extent, by infection by Human Papilloma Virus (HPV), which is the main risk factor for oropharynx squamous cell carcinoma (OPSCC).

Current strategies implemented for OSCC include surgery, radiotherapy and chemotherapy. Late diagnosis, aggressive phenotype, recurrences and resistance to therapies are responsible for a poor 5-year overall survival rate, despite modern advances in diagnosis and treatment [1].

Recent clinical studies report that the use of metformin, an anti-diabetic drug, reduces the risk of head and neck squamous cell carcinoma in diabetic smokers and alcohol consumers compared to nondiabetic ones and this drug has also been shown to improve the prognosis of cancer patients, that present less distant metastasis and better survival outcomes [2].

From recent studies, Chromatin Assembly Factor - 1 (CAF-1), in particular the major subunits p60 and p150 of this heterotrimeric protein complex, crucial for assembling, replication and reparation of the DNA, appear to be promising diagnostic [3] and prognostic [4] factors in OSCC patients.

AIM

This study aimed to evaluate the effect of metformin on the aggressive behavior of OSCC cell lines and to verify whether this drug may affect CAF-1 expression.

METHODS

HPV- (CAL27) and HPV+ (SCC154) OSCC cell lines were treated with metformin 10 mM (based on MTT assay) and then the effect on their aggressiveness was evaluated by colony forming assay (Fig. 1), wound healing assay (Fig. 2), invasion assay (Fig. 3) and by immunofluorescence of epithelial-mesenchymal transition markers, E-cadherin and N-cadherin (Fig. 4) for different times.

The expression of CAF-1 major subunits, p150 and p60 was visualized by western blot (Fig. 5) of lysates of cells treated with metformin from 24 to 96 hours of treatment.

RESULTS

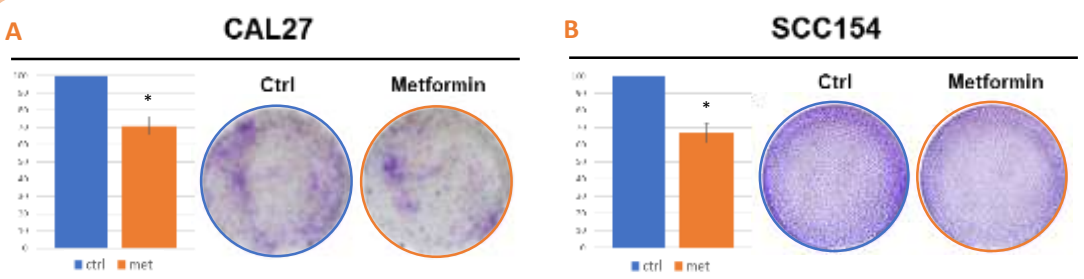

Fig. 1 Cells treated with metformin for 24h show a **lower ability to form colonies** compared to controls in 10 days of incubation.

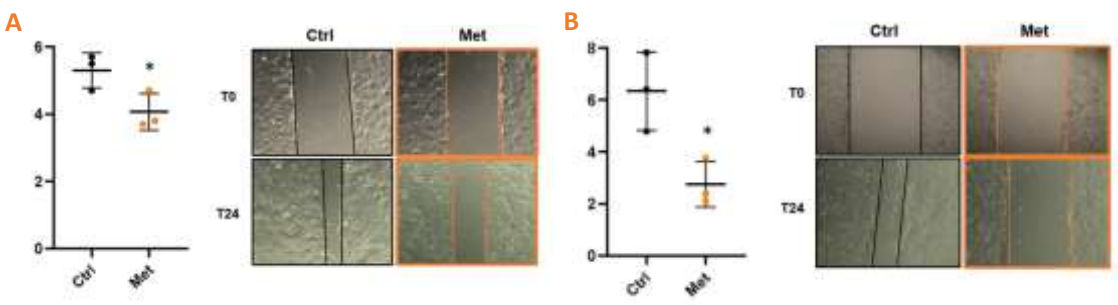

Fig. 2 In presence of metformin, cells repair the wound more slowly than controls, due to the **decreased migration capacity**.

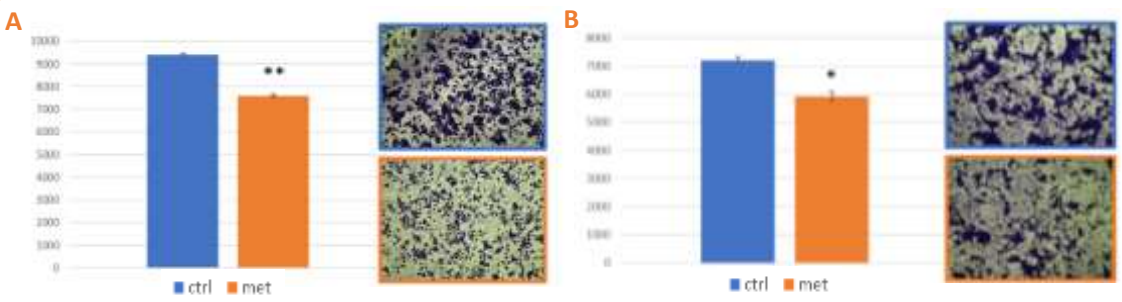

Fig. 3 Residual cells in the matrigel matrix are significantly fewer after 24h of metformin treatment, showing a **reduction of invasion capacity**.

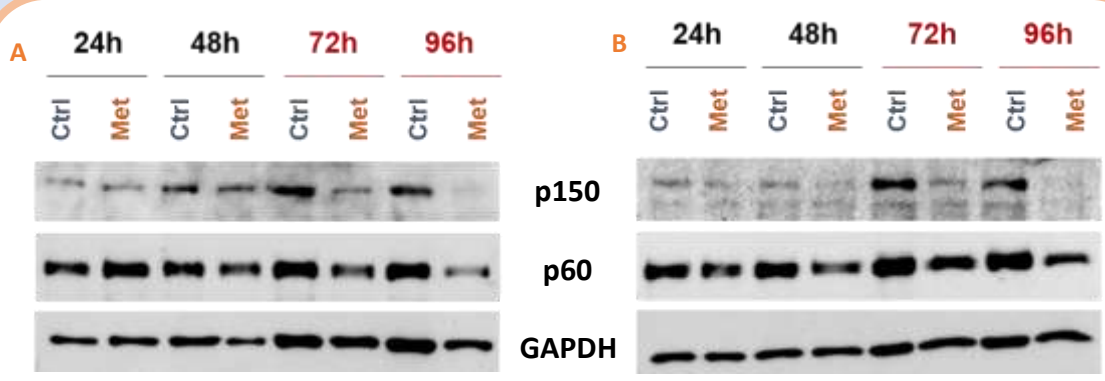

Fig. 5 Unexpectedly **expression of CAF-1** major subunits, p150 and p60, is **suppressed** under effect of metformin in a time-dependent way.

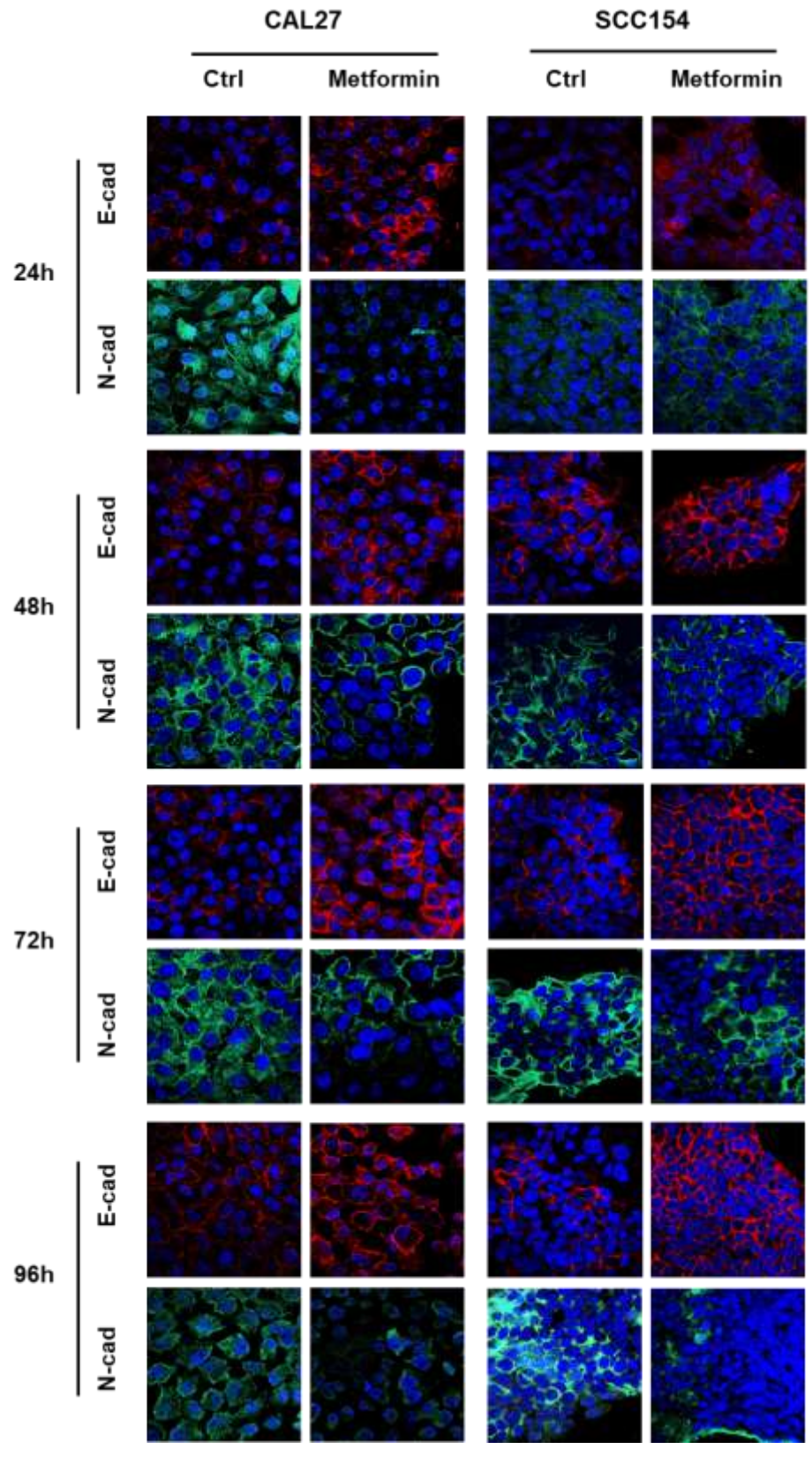

Fig. 4 Immunofluorescence shows the **reversal of the epithelial-mesenchymal transition** of these cells in presence of metformin, with an increase in E-cadherin and a decrease in N-cadherin.

CONCLUSIONS & FUTURE PERSPECTIVES

In conclusion:

- ✓ Metformin reduces the aggressive phenotype of CAL27 (HPV-) and SCC154 (HPV+) cell lines, by decreasing their ability to form colonies (Fig. 1), migrate (Fig. 2) and invade (Fig. 3) and by reversing the expression of epithelial-mesenchymal transition markers (Fig. 4);
- ✓ Metformin downmodulates the expression of CAF-1 major subunits (Fig. 5), recently related to tumor aggressiveness.

Next experiments:

- ➢ Testing the effects of metformin on other cell lines and primary cultures;
- ➢ Evaluating the effect of metformin in combination with radiotherapy on cells;
- ➢ Experimenting response to metformin in overexpressing CAF-1 cell lines, to elucidate CAF-1 involvement.

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