Expression of fibroblast activation protein-α in human glioblastoma and its effect on glioma cell adhesion and invasion

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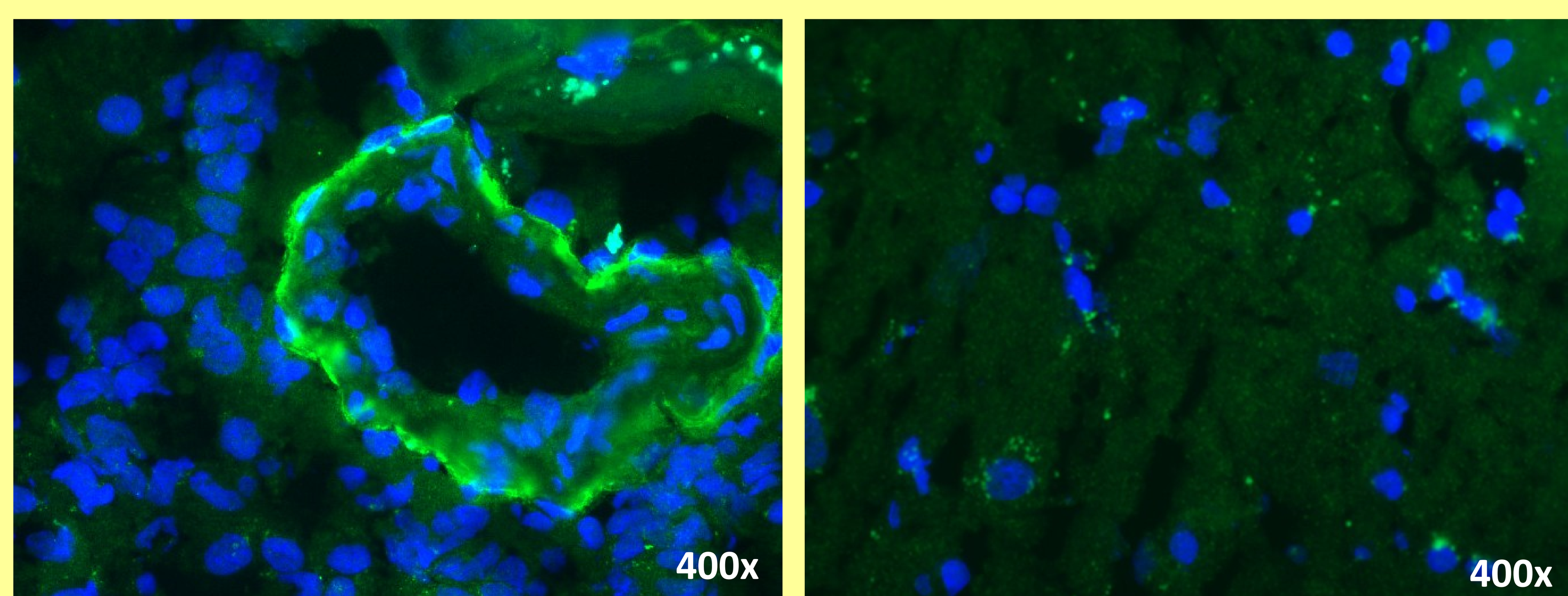
Introduction

Malignant gliomas are aggressive tumours with invasive growth due to aberrant interactions with extracelular matrix and characteristic spreading along the white matter tracts, blood vessels and the subpial space. Fibroblast activation protein-α (EC 3.4.21.-; FAP) is a dual specificity serine protease with a post proline dipeptidyl exopeptidic and collagenolytic endopeptidic activity. FAP, processing both structural and regulatory peptides is suggested to participate in the degradation of the extracellular matrix, tissue remodelling and cancer cell invasion. Increased FAP mRNA expression was previously demonstrated in human glioblastoma [1], but its role in glioma progression remains largely unknown.

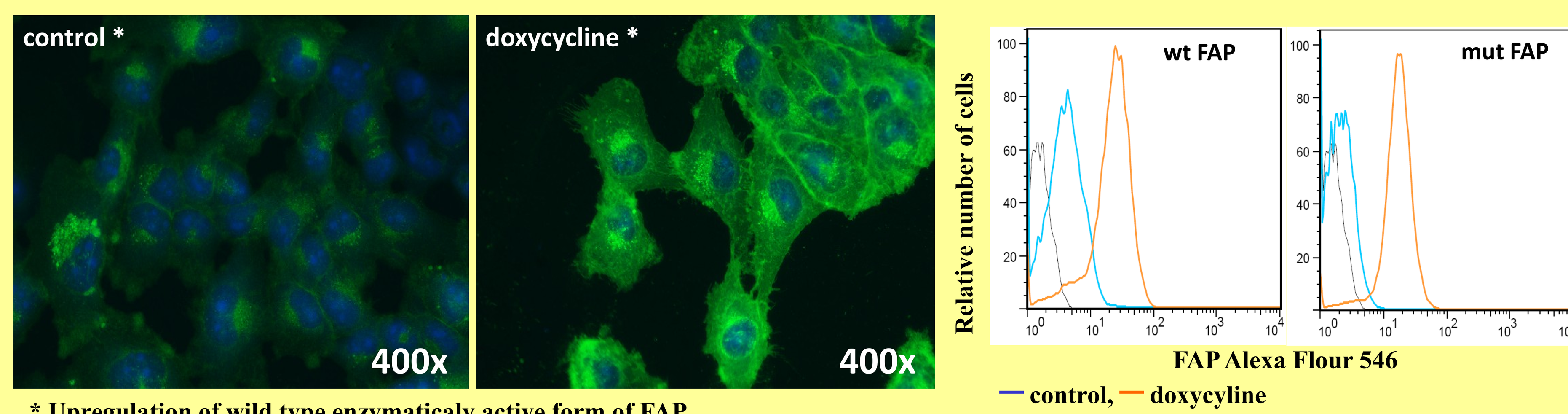
The aim of the study is to analyze the staining pattern of FAP in human glioblastoma compared to the non-malignant brain, and determine the effect of FAP on glioma cell adhesion, migration and invasion.

Results

I. Localization of FAP positive cells predominantly around blood vessels in human glioblastomas (A) compared to non-malignant brain (B).

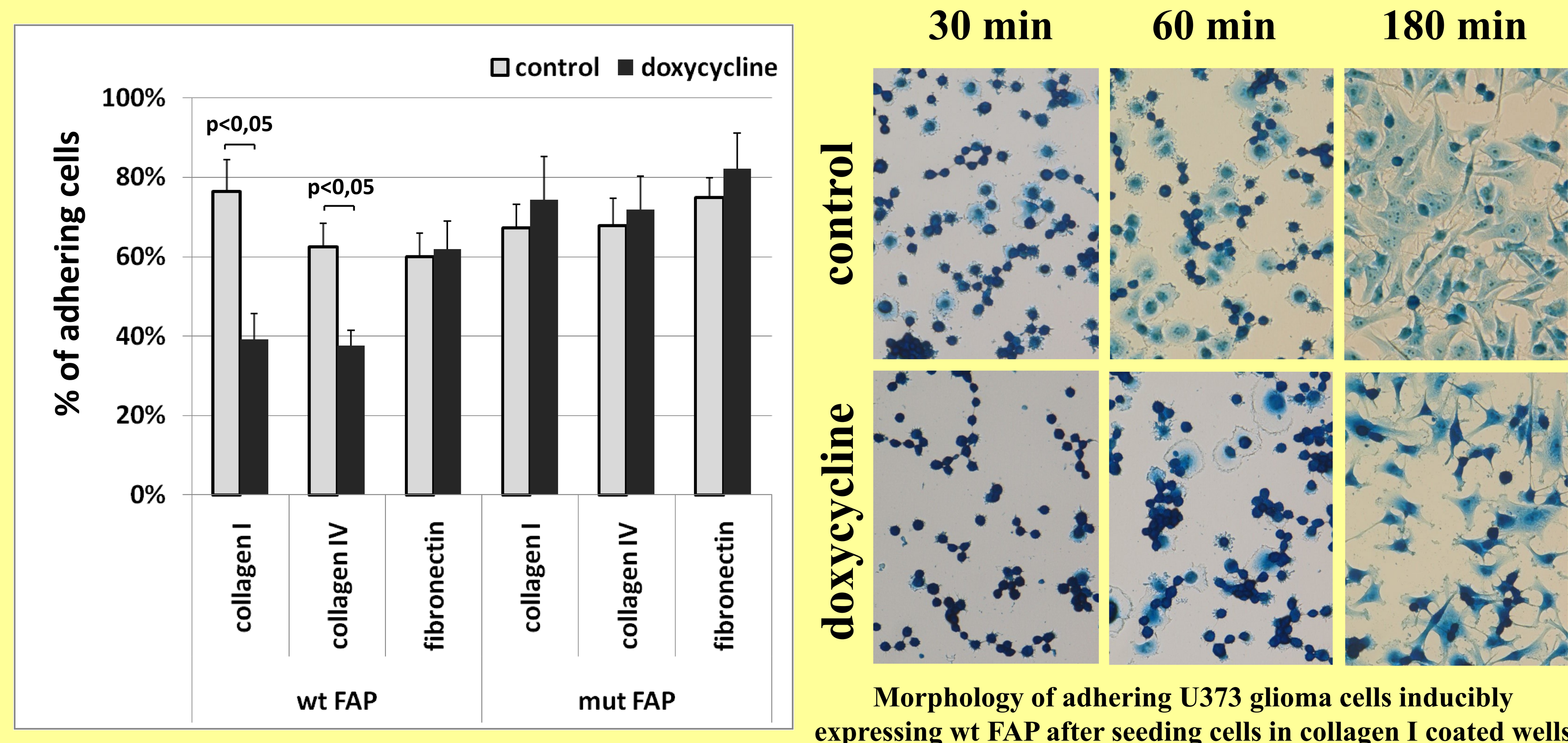


II. Doxycycline inducible expression of transgenic wild type (wt FAP) and enzymatically inactive mutant FAP (mut FAP) in the human glioma cell line U373.



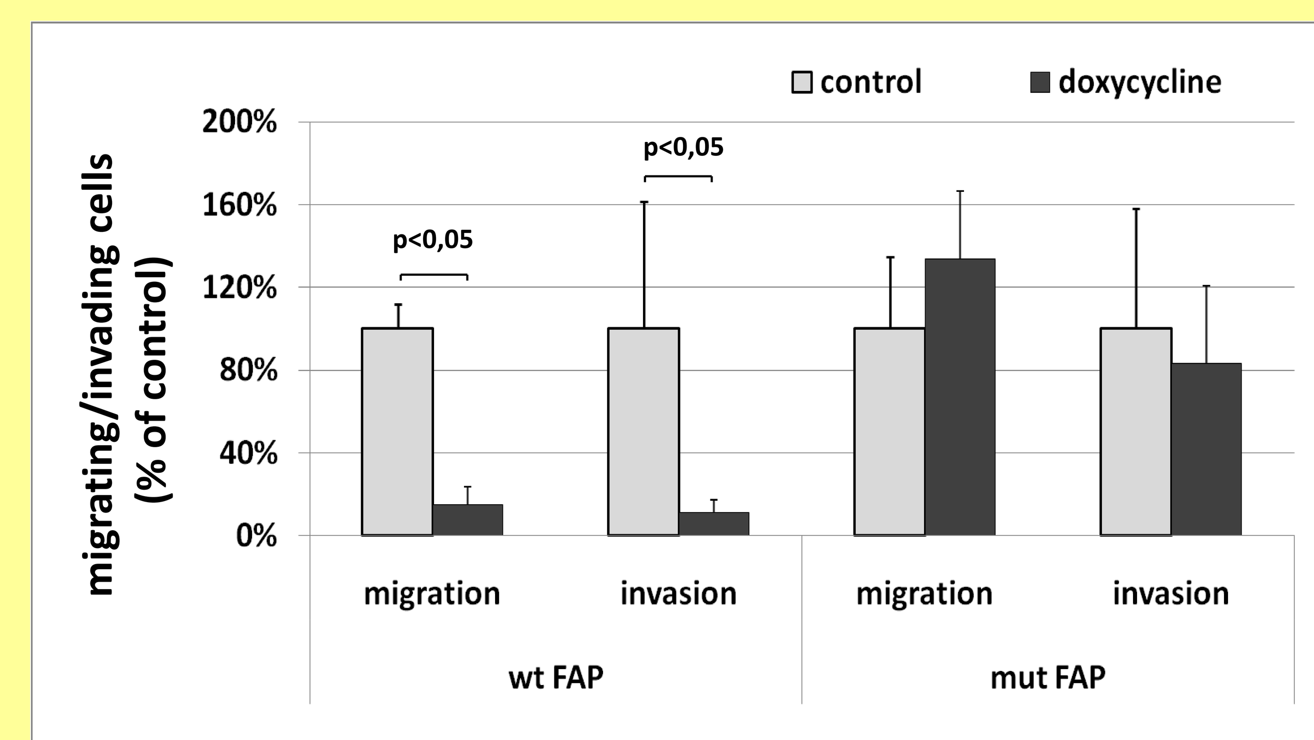
* Upregulation of wild type enzymaticaly active form of FAP

III. Transgenic wild type FAP negatively influences the adhesion and spreading of U373 glioma cell on collagen I and IV.

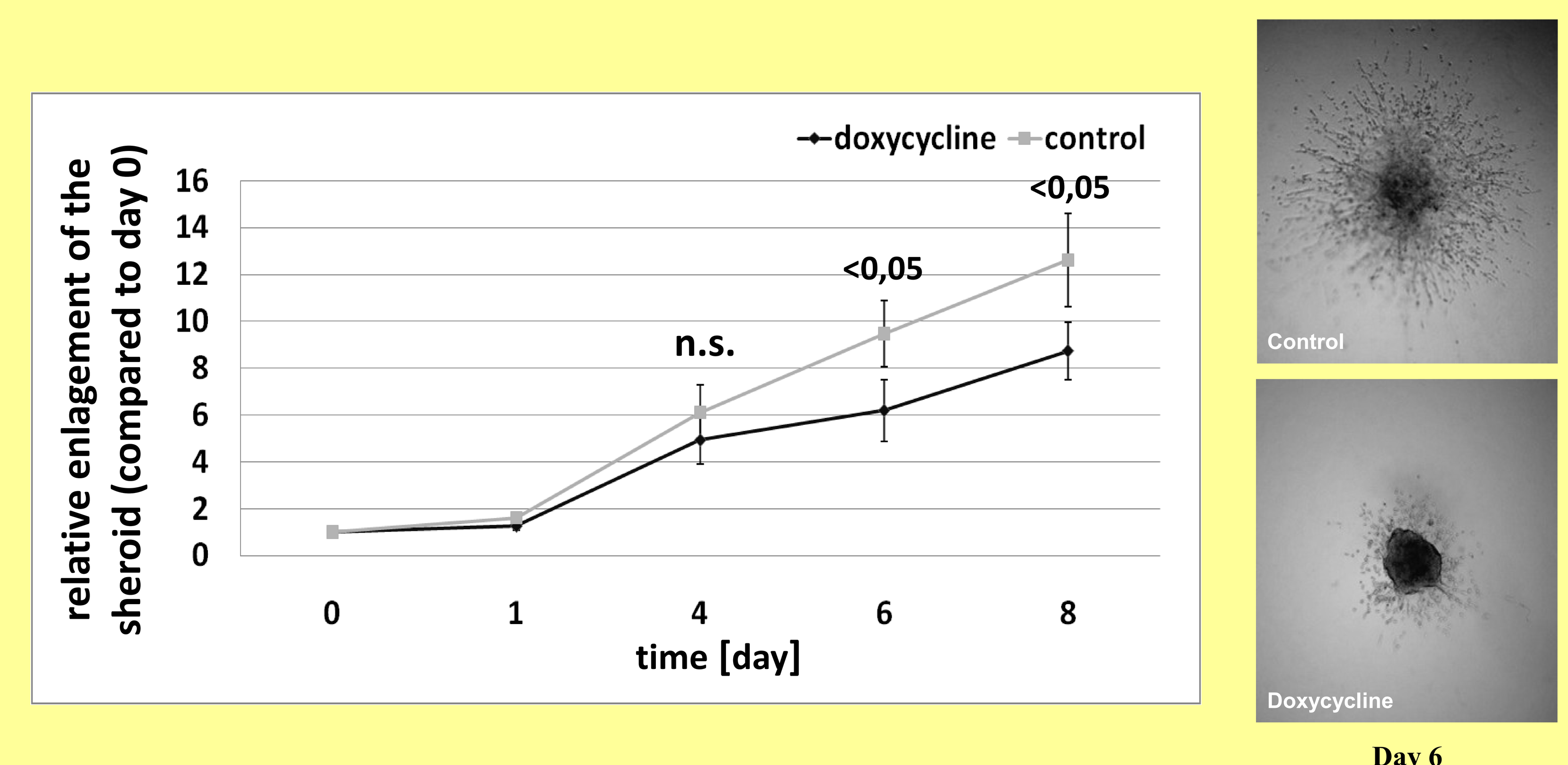


Morphology of adhering U373 glioma cells inducibly expressing wt FAP after seeding cells in collagen I coated wells

IV. Transgenic wild type FAP decreases the migration and invasion through matrigel in U373 glioma cells.



V. Transgenic wild type FAP negatively influences the ability of U373 to invade collagen I in a spheroid invasion model.



Day 6

Conclusions

- **FAP is predominantly localized in the close vicinity of blood vessels in the human glioblastoma.**
- **Overexpression of transgenic wild type FAP in U373 glioma cells leads to decreased migration and invasion, as well as cell adhesion and spreading on collagen I and IV, but not fibronectin.**
- **FAP may influence the interaction of glioma cells with specific components of the surrounding extracellular matrix via its enzymatic activity.**

Matherial and Methods:

U373 cells (ATCC, Middlesex, UK) were transfected with the wild type and mutant (active site Ser624→Ala) FAP using the doxycycline inducible TetOn system (Clontech, USA) according to the manufactorer's instructions. The cells were cultured under standard conditions.

Immunodetection of FAP was performed using the rat monoclonal anti human FAP antibodies (clones D8 and D28, Vitatex, USA).

Adhesion assays were performed in 96WP coated with collagen I, IV or fibronectin (Sigma, CR). Cell migration and invasion was evaluated using the modified Boyden chamber assay using cell culture inserts with 8 μm pores.

Spheroids were formed using the „hanging drop“ method and embeded in collagen I. Cell invasion was assessed by measuring the area covered with invading cells.

References:

Stremenova, J., Krepela, E., Mares, V., Trim, J., Dbaly, V., Marek, J., Vanickova, z., Lisa, V., Yea, C., Sedo, A. (2007) Expression and enzymatic activity of dipeptidyl peptidase-IV in human astrocytic tumours are associated with tumour grade. Int. J. Oncol. 31, 785-792.

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