The identification of nuclear αvβ3 integrin in ovarian cancer: non- paradigmal localization with cancer promoting actions

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INTRODUCTION

High-grade serous ovarian cancer (HGSOC) is a highly aggressive gynecological malignancy. The source of HGSOC is the fallopian tube (FT) epithelium secretory cells. Emerging evidence has shown the translocation of central proteins from the cell membrane to the nucleus in cancer. The membrane receptor **αvβ3 integrin** is amply expressed in HGSOC and involved in disease progression, however its nuclear localization was never demonstrated.

OBJECTIVES

Our research aims in HGSOC were to:

- Identify αvβ3 **nuclear expression**
- Study αvβ3 **nuclear functions**
- Study αvβ3 **nuclear interactions.**

METHODS

> Cell culture: HGSOC (OVCAR3, KURAMOCHI, JHOS4), HEK293β3 & OVCAR3 β3-NLS transfected cells, normal ovaries/FT (CHO-K1, FT282) and β3-NLS enriched OVCAR3 cells
> Human tissue samples: Fresh/archived from patients (Helsinki approved)

METHODS (Continued)

> Western blot (WB): For αvβ3/other proteins
> Confocal Immunofluorescence (IF) microscopy
> αvβ3 Co-Immunoprecipitation (Co-IP): For nuclear extracts using anti αvβ3 antibody

RESULTS

We identified nuclear αvβ3 in HGSOC cells (**Fig.1A**) and tissues from patients (**Fig.1B,** left panel), but not in normal ovaries and FT cells (**Fig.1C**) and tissues (**Fig1.B,** right panel). Results were confirmed by confocal microscopy for HGS cells **(Fig.2A**) and tissues (**Fig.2B**). Nuclear αvβ3 enriched HGS cells demonstrated induced oncogenic signaling (**Fig.3A**) , proliferation (**Fig.3B**), cell cycle (**Fig.3C**), intact colony formation and inhibited migration (data not shown). Proteomics analyses revealed a network of nuclear αvβ3-bound proteins, with distinct clustering of HGSOC cells (**Fig.4A-B**) and more shared proteins (**Fig.4C**) compared to αvβ3-transfected normal HEK293 cells. Reverse co-IP confirmed nuclear αvβ3 binding of several key cancer-relevant proteins (**Fig.4D**).

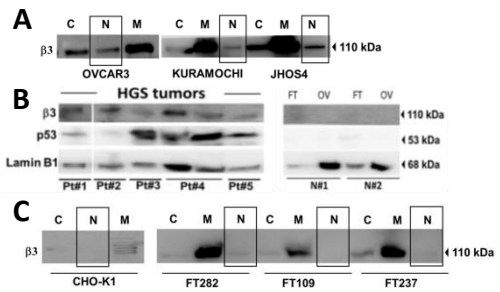

Fig. 1. Nuclear αvβ3 in HGSOC cells (A) WB of Cellular fractions (C cytosolic, M membrane, N nuclear) and **(B)** Nuclear fractions from tumor (pt#1-5) and normal (N#1,2) tissues. LaminB1 confirms nuclear fractions **(C)** Fractions from normal ovary (CHO-K1) and FT (FT282, FT109, FT237) cell lines

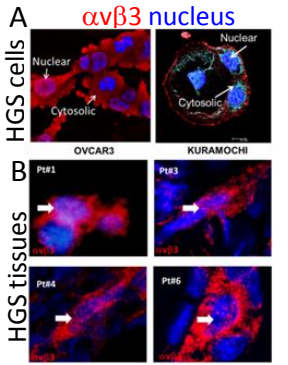

Fig. 2. Nuclear αvβ3 confocal IF microscopy in HGSOC (A) cells and **(B)** human tissues

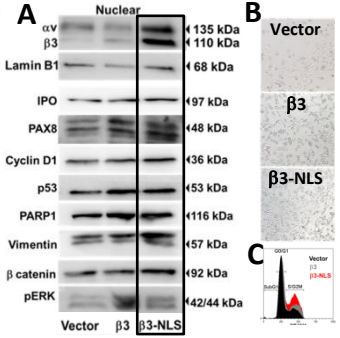

Fig. 3. Nuclear enriched αvβ3 (β3-NLS) in HGSOC cells (A) WB **(B)** Microscopy **(C)** Cell cycle

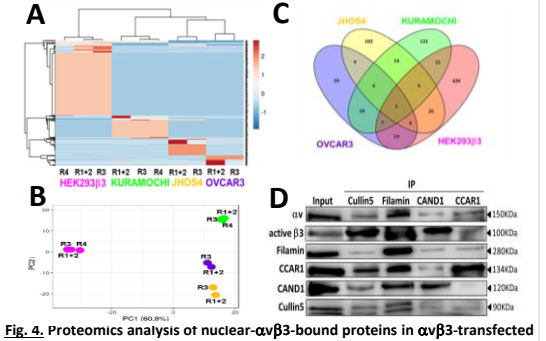

Fig. 4. Proteomics analysis of nuclear-αvβ3-bound proteins in αvβ3-transfected HEK293 cells and HGSOC cells. (A) Hierarchical clustering, (ClustVis web tool). **(B)** Principal component analysis (PCA). **(C)** Shared proteins (Venny tool). **(D)** Reverse co-IP: for nuclear αvβ3 Interacting proteins in β3-NLS OVCAR3 cells

CONCLUSIONS

We identified a nuclear αvβ3 integrin reservoir in ovarian cancer with a proposed role and interactions that may be distinct from its plasma membrane actions. This unique biological mechanism, which has been largely overlooked, adds to the multifaceted activities reported for αvβ3 and may lead to improved understanding of the molecular basis of ovarian cancer