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

Seraya-Bareket et al.

Supplementary Figure 1: (A) ImageStream similarity score histograms for the various channel combinations in OVCAR3 and KURAMOCHI. A high similarity score between the green (extracellular αvβ3) and the blue (cell nucleus) channels is depicted (Average±STDEV) while the other channel combinations present no significant similarity. (B-D) Controls for Western blots Western blot analysis of fraction purity and loading controls for the various cell models. Western blots analysis of Na+K+, Lamin B1, GAPDH or tubulin in cytosolic (C), nuclear (N) and membrane (M) protein fractions in (B) OVCAR3, KURAMOCHI and JHOS4 HGS cell lines and (C) CHO-K1 normal ovary cells (D) Loading control (Ponceau S) and fraction purity (GAPDH) in FT282, FT109 and FT237 fallopian tube cell lines. Representative blots are shown. (E) Quantification of band intensity of the WB presented in Figure 1 as ratio of target protein, normalized to loading. Densitometry is expressed as percentage compared with CHO-K1 (considered as 100%). Values are means ± STE. (F) Controls for the confocal microscopy. IgG isotype controls for the various staining methods in the HGSOC cells. Merged channels are shown.
Supplementary Figure 2: Summary of the HGSOC tissue control assessment. (A) IHC analysis for p53, pax8 and p16 in tumor/normal regions in the ovary and FT tissues from HGSOC patients (B) FT and ovarian tissues from normal controls assessed for p53. (C) Western blots analysis of Na+K+ and GAPDH in nuclear fractions from patients #1-5. (D) Quantification of band intensity of the WB presented in Figure 2A as ratio of target protein normalized to loading. Values are means ± STE. (E) FFPE images of HGS ovarian cancer tissues from patients #2, #5 and #9 stained for αvβ3 by IHC. Nuclear integrin is indicated by a black arrow. (F) IHC images of tumor free fallopian tube tissue from patient #1 stained negative for p53 and for αvβ3. (G) IHC images of normal fallopian tube tissues stained negative for p53 and for αvβ3. X40 objectives. Scale bars: 20µm.
Supplementary Figure 3: Analysis of native and αvβ3 transfected HEK293 cells. 

(A) Flow cytometry analysis of αvβ3 integrin in non-transfected cells and αvβ1 or αvβ3 transfected cells (B) Western blots analysis of tubulin to determine protein extraction from equal amount of cells (C) Fraction purity by WB in cytosolic (C), nuclear (N) and membrane (M) protein fractions from native and αvβ3 -transfected HEK293 cells. GRP78, extracted with other membrane proteins, was used as an ER marker and tubulin, as a cytosolic marker (D) Western blots analysis of Na+K+, Lamin B1 and tubulin in cytosolic and membrane fraction (C+M) and nuclear fraction (N) in HEK293 and HEK293β3 (E) Quantification of band intensity of the WB presented in Figure 3A as ratio of target protein, normalized to loading. Values are means ± STE. (F) A high similarity score between the green (intracellular αvβ3) and the blue (cell nucleus) channels is depicted (Average±STDEV) while the other channel combinations present no significant similarity in the HEK293β3 cells. (G) Representative Western blots from αvβ3 integrin in αvβ3-tranfected HEK293 cells following IP using anti αvβ3 dimer antibody or an isotype control IgG antibody. Protein input, flow-through, wash and pull down fractions are depicted (H) Representative Western blots from αvβ3 integrin in αvβ3-tranfected and native HEK293 cells following IP using anti αvβ3 dimer antibody. Protein input and pull down fractions are depicted.
Supplementary Figure 4: Western blot analysis of nuclear fractions extracted from OVCAR3 cells transfected with an empty vector, β3 integrin and NLS-modified β3 integrin. Western blots analysis for (A) Nuclear fraction purity using Na+K+ and GAPDH controls. (B) Representative Western blots from OVCAR3 cells transfected with β3-NLS vector and HEK293β3 cells transfected with the native β3 following IP from nuclear and non-nuclear fractions using anti αvβ3 dimer or IgG control antibodies. Protein input and pull down fractions are depicted for actin and talin in the nuclear and non-nuclear fractions (C-F) Lamin B1 loading controls.
Supplementary Figure 5: Interaction network between the proteins which complexed with the nuclear αvβ3 in the various cell models. The online STRING tool was used to generate images (https://string-db.org).