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Supplementary Materials for

Migratory behaviour of tumor cells: a scanning electron microscopy study

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Published on Ann Ist Super Sanità 2015 Vol. 51, No. 2 DOI: 10.4415/ANN_15_02_12

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MCF-7 ADR MCF-7 ADR LoVo ADR LoVo ADR LoVo WT LoVo WT MCF-7 WT MCF-7WT

Membranes of modified Boyden chamber assay stained with crystal violet. Left panels migration assay. Right panels invasion assay.

Supplementary data 1

Membranes of modified Boyden chamber assay stained with crystal violet. Left panels migration assay. Right panels invasion assay.



Scanning electron microscopy observations performed on the upper side (a, c, e, g, i, k, m) and on the lower side (**b**, **d**, **f**, **h**, **j**, **l**, **n**) of the filter during the migration process. The observations performed on the upper side provide evidence that MCF-7 WT (a), LN229 (g) and LoVo ADR (m) cells adopted a "collective" behaviour, whereas MDA-MB-231(e), C6 (i) and LoVo WT (k) cells adopted a "individual" behaviour. MCF-7 ADR (c) cells showed a "mixed" behaviour. Cells that adopt an individual behaviour tend to separate from the rest of the cell population and to pass through the pores individually. In the "collective" behaviour clusters of cells move closely linked each other. In these groups of cells it can be identified a leader (arrows) dragging the other cells. These leader cells generate the traction force necessary for the migration of the group, through the activity of pseudopodia, pulling behind resting cells. Observations performed on the lower side of porous membranes, confirmed data obtained by the quantitative analysis. MCF-7 WT cells samples (b) showed the smallest number of migrated cells on the lower side of the filter. In MCF-7 ADR cell samples (d) the number of migrated cells increased, while migrated MDA-MB-231 cells covered almost the whole area of the lower side of the filter (f). In C6 cell sample (j) numerous cells completed the migration process, in contrast with LN229 cells that moved more slowly (h). Finally, LoVo WT samples (I) displayed a number of cells lower than their resistant counterpart LoVo ADR cells (n).



Scanning electron microscopy observations performed on the upper side (a, c, e, g, i, k, m) and on the lower side (b, d, f, h, j, l, n) of the filter during the invasion process in presence of Matrigel[™]. The observations performed on the upper side provide evidence of "individual" or "collective" behaviour adopted by tumour cells. In the presence of a film of Matrigel™, MCF-7 WT (a), LoVo WT (k) and LoVo ADR (m) cells adopted a "collective" behaviour, whereas MDA-MB-231 (e), LN229 (g), and C6 (i) cells adopted an "individual" behaviour. MCF-7 ADR (c) cells showed a "mixed" behaviour. The observations on the lower side provide information on the type of tumour cell-extracellular matrix interactions during the invasion process. MCF-7 WT (b), MCF-7 ADR (d), MDA-MB-231 (f) cells adopted a "mesenchymal" behaviour whereas, C6) (j), LoVo WT (I) and LoVo ADR (n) adopted an "amoeboid-like" behaviour. Cells that adopt a "mesenchymal" behaviour showed an intense proteolytic activity focused around invadopodia. Focused proteolysis was due to proteases strongly concentrated near the binding sites between integrins and extracellular matrix. Cells that adopt an "amoeboid" behaviour penetrated the fibers of Matrigel[™] without degrading it, but infiltrating and invading the extracellular matrix. However, there are cells that show a "mixed" behaviour (LN229, h): while in some areas matrix degradation was observed, suggesting that cells recurred to an invasion of mesenchymal type, in other areas cells appear to infiltrate through the mesh of the matrix in "ameboid-like" manner.

SUPPLEMENTARY MATERIALS



Supplementary data 5

Flow cytometry analysis of CD44, E-cadherin and VLA2 proteins on: (a) MCF7 WT, (b) MCF7 ADR, (c) M14 WT, (d) M14 ADR, (e) LoVo WT, (f) LoVo ADR, (g) MDA-MB-231, (h) LN229 cells.





Flow cytometry analysis of VLA5 protein on: (a) MCF7 WT, (b) MCF7 ADR, (c) M14 WT, (d) M14 ADR, (e) LoVo WT, (f) LoVo ADR, (g) MDA-MB-231, (h) LN229 cells.