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Immunophenotypic characterization of canine histiocytic sarcoma cells with and without canine distemper virus infection

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Adhesion to basement membranes and extracellular matrix represents one of the main steps resulting in an invasive phenotype of tumor cells5. Oncolytic viruses are believed to be able to interfere with these processes efficiently resulting in a less malignant phenotype of tumor cells3. Considering the main role that the expression of cell-surface associated molecules play in tumor invasion1,2,4,5,6, it was the aim of the present study to verify if a CDV Onderstepoort (CDV-Ond) infection of canine histiocytic sarcoma cells (DH82 cells) modulates the expression of adhesion and signalling molecules that could interfere with their invasive phenotype in an effort to step forward on considering CDV-Ond as a virus-mediated therapy for histiocytic sarcomas.

Therefore we focus on the expression of some integrins, immunoglobulin superfamily members and other important molecules for cell-cell and cell-matrix interactions, as well as for cell integrity, functionality and immunological properties.

Fluorescence activated cell sorting for:

• **Adhesion**, signalling and immunocompetence, where cells were tested for CD1c, CD11b, CD11c, CD14, CD18, CD44, CD45,CD45RA, CD80, CD86, ICAM1, MHCI and MHCII antibodies, subsequently labelled with gamPE, rarPE or scFITC fluorochromes and measured for fluorescence intensity reading 10000 events with FACS flow cytometer (BD). Native cells and respective mouse IgG1, IgG2 and IgG2a and rat: IgG2a and IgG2b isotypes were used as negative controls. Cell-Quest Pro® software (BD) and T-test statistical analysis was used for data evaluation.

• **CDV infection assessment**, using an antibody directed against CDV nucleoprotein and determining fluorescence as described above.

• **Determination of phagocytic activity**, using FITC- labelled Staphylococcus sp. bacteria and measuring the percentage of fluorescence.

Cell lines:

• Canine histiocytic sarcoma cells, DH82 cell line.
• CDV-Ond-persistently infected DH82 cells.

Migration Studies

were performed with CDV-Ond infected and uninfected DH82 cells in a two-chamber type transwell system with or without a Matrigel® coating and counting the migrated cells in the lower chamber after 1, 2 and 3 days.

Result 1

Fluorescence activated cell sorting Adhesion and signalling

CD11a, CD11b, CD11c and CD14 adhesion molecules showed. statistically significant up-regulation in CDV-Ond infected cells. Similarly CD80, co-stimulatory molecule, was up-regulated (FIG.1).

Immunocompetence

MHC I was highly expressed in both cell lines, whereas they nearly lacked MHC II expression (FIG.1).

Phagocytosis

Functionality test of CDV-Ond infected and uninfected DH82 cells showed up to 98 % of phagocytic activity (data not shown) and reveals no differences between both cell lines.

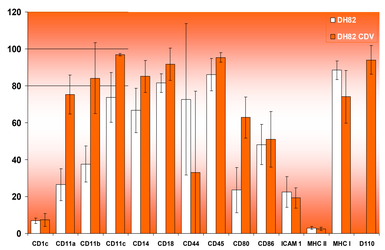

Fig. 1: Ciliary activity of infected TOCs. (A) Chicken TOCs infected by 104 pfu/ring of egg-grown A/chicken/SaudiArabia/CP7/1998 (H9N2) or the H9N2 virus passaged four times in chicken TOCs. (B) Turkey TOCs infected by 104 pfu/ring of A/chicken/SaudiArabia/CP7/1998 (H9N2) or the H9N2 virus passaged four times in turkey TOCs.
The ciliary activity of the TOCs was monitored under a light microscope and served as a criterion for viability.

Result 2

Invasiveness

CDV-Ond persistently infected DH82 cells sparsely migrate to the lower chambers of the transwell system after 24 and 48 hours post-seeding and a 4–fold lower amount of cells are found after 72 hours in contrast with non-infected cells corresponding to control inserts. In Matrigel coated inserts unremarkable differences were shown between both cell groups after 24 hours but statistically significant differences after 2 and 3 days (FIG.2).

Uninfected DH82 cells

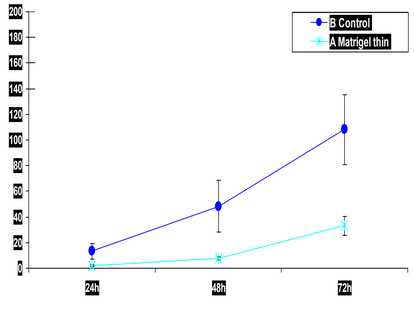

Fig. 3: Ciliary activity of infected TOCs. (A) Chicken TOCs infected by 104 pfu/ring of egg-grown A/chicken/SaudiArabia/CP7/1998 (H9N2) or the H9N2 virus passaged four times in chicken TOCs.

CDV-Ond DH82 cells

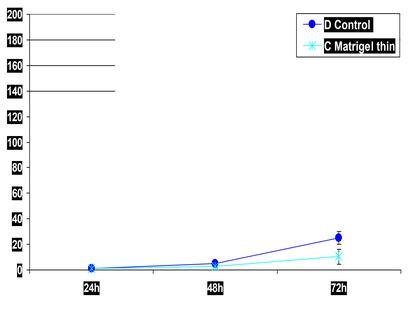

Fig. 2: Ciliary activity of infected TOCs. (A) Chicken TOCs infected by 104 pfu/ring of egg-grown A/chicken/SaudiArabia/CP7/1998 (H9N2) or the H9N2 virus passaged four times in chicken TOCs.

Discussion

The up-regulation of adhesion molecules expressed, following CDV Onderstepoort infection of histiocytic sarcoma cells, is suspected to alter the necessary adhesion / anti-adhesion duality required for cell tumor motility and invasion, resulting in a reduced invasive phenotype as shown in Matrigel invasion assays.

References

[1] Friedl P, Bröcker EB (2000): The biology of cell locomotion within three-dimensional extracellular matrix. CMLS Cell Mol Life Sci;57: 41-64.[2] Jothy S, Munro SB, LeDuy L, McClure D, Blaschuk OW (1995): Adhesion or anti-adhesion in cancer: what matters
more?. Cancer and Metastasis Reviews; 14: 363-376.[3] Prestwich RJ, Harrington KJ, Pandha HS, Vile RG, Melcher AA, Errington F (2008): Oncolytic viruses: a novel form of immunotherapy. Expert Rev Anticancer Ther; 8 (10): 1581-1588.[4] Schmidt S, Friedl P
(2010): Interstitial cell migration: integrin-dependent and alternative adhesion mechanisms. Cell Tissue Res; 339: 83-92.[5] Stetler-Stevenson WG, Aznavoorian S, Liotta LA (1993): Tumor cell interactions with the extracellular matrix during invasion and metastasis. Cell Biol;
9: 541-73.[6] Zetter BR (1993) Adhesion molecules in tumor metastasis. Semin Cancer Biol; 4(4): 219-29.

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Abbreviations

CDV-Ond- Canine Distemper Virus, Onderstepoort strain; FACS- Fluorescence Activated Cell Sorting; gamPE- goat anti mouse PhycoErythrin; Ig- Immunoglobulin; MOI- Multiplicity Of Infection; scFITC- streptavidin conjugated Fluorescein IsoThioCyanate;