EFFECTS OF EXOSOMES RELEASED FROM K562 CML CELL LINE ON γδ T CELLS FUNCTION.

L. Saieva [1] , S. Taverna [1], A. Flugy [1], G. De Leo [1], R. Alessandro [1].

[1]Dipartimento di Biopatologia e Biotecnologie mediche e forensi, Sezione di Biologia e Genetica, Università di Palermo, via Divisi 83, 90133, Palermo, Italy. email: laura.saieva@unipa.it

Chronic myeloid leukemia is a clonal hematopoietic stem cell disorder in which leukemic cells display a distinctive shortened chromosome, the Philadelphia chromosome, generated from a reciprocal t(9:22) translocation[1]. This translocation results in the head-to-tail fusion of the breakpoint cluster region (BCR) gene on chromosome 22 with the ABL proto-oncogene on chromosome 9. The encoded chimeric Bcr-Abl oncoprotein exhibits constitutive tyrosine kinase activity leading to its autophosphorylation and activation of multiple signaling molecules[2]. As a consequence, Bcr-Abl leads to increased proliferation, inhibition of apoptosis and altered adhesion of leukemic blasts to bone marrow (BM) microenvironment. This is characterized by different populations of accessory stromal and T cells along with other factors including extracellular matrix and vessels. Microvesicles shed by cancer cells, released in the tumor-microenvironment, can bind to target cells, transfer signals between different cells to mediate biological functions and may have a key role in disease progression. Recently, attention is being focused on cell-cell communication that involves membrane fragments called exosomes. Exosomes are small vesicles of 40-100 nm diameter of endosomal origin which are secreted from different cell types including cancer cells[3]. Several data in the last years have showed that exosomes are messengers in intercellular communication and that tumor cells can use these vesicles to affect immune function. Several studies reported activatory[4] and inhibitory[5] effects of cancer-exosomes on lymphocytes; differences are probably dependent on the exosomes molecular composition. Mounting evidence is pointing to exosomes as major participants in immune evasion through the expression of a large array of bioactive molecules. We studied the effects of exosomes shed by Chronic Myelogenous Leukemia (CML) cell line, K562, on the function of γδ lymphocytes to better understand the interaction between cancer exosomes and the immune system. γδ T cells act as first-line defenders in innate immune responses against infected and tumor cells. Vγ9Vδ2 T cells are activated by metabolites of mevalonate pathway (such as isopentenyl pyrophosphate, IPP), and non-mevalonate pathway (such as E-4-hydroxy-3-methyl-but-2-enyl pyrophosphate, HMBPP)[6] or nitrogen-containing bisphosphonate (N-BPs) such as zoledronate[7]. Vγ9Vδ2 T cells can recognize and exert cytotoxicity against tumor cells in various ways. These mechanisms include MICA/B or UL16 binding protein (ULBP) interactions with NKG2D[8] or the recognition of metabolites of the mevalonate pathway to sense cells that are metabolically altered. γδ T cells exhibit a potent MHC-unrestricted lytic activity against several tumor cells and are able to kill CML cells pretreated with zoledronic acid. Zoledronic acid is a syntethic aminobiphosphonate able to activate γδ T cells, augments the anti-Ph^+ leukemia activity of imatinib *in vitro* and *in vivo* and inhibits proliferation and induces apoptosis of imatinib-resistant CML cells. Furthermore, we treated K562 with Zoledronic Acid (ZA) to evaluate the effects of this drug on the release of exosomes. K562 cells treated with ZA release a less amount of exosomes compared to untreated cells. CML exosomes are able to induce a dose-dependent down-regulation of activation markers, CD69 and CD25, and NKG2D expression when γδ T cells are activated with HMBPP and IL2. Exosomes affect also the IFN-γ and TNF-α release by γδ T cells, suggesting that exosomes from CML have an inhibitory effect on lymphocytes function. These initial findings on the role of CML exosomes on γδ T cell shed new light on the ability of cancer cells to hide from immune system through an exosomes-mediated mechanism.

Bibliografia:

1. Rowley J. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973
2. Sonoyama J. et al. Functional cooperation among Ras, STAT5, and phosphatidylinositol 3-kinase is required for full oncogenic activities of BCR/ABL in K562 cells. J Biol Chem 2002
3. Wolfers J. et al. Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. *Nature Med.* 2001
4. Gastpar R. et al. Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. Cancer Res 2005
5. Liu C. et al.Murine mammary carcinoma exosomes promote tumor growth by suppression of NK cell function. J Immunol 2006
6. Altincicek B. et al. Cutting Edge: Human gd T Cells Are Activated by Intermediates of the 2-*C*methyl-D-erythritol 4-phosphate Pathway of Isoprenoid Biosynthesis. J. Immunol 2001
7. Caccamo N et al. Aminobisphosphonate-activated gamma delta T cells in immunotherapy of cancer: doubts no more. *Expert Opin Biol Ther.* 2008
8. Wrobel P et al. Lysis of a broad range of epithelial tumour cells by human γδ T cells:involvement of NKG2D ligands and T-cell receptor- versus NKG2Ddependent recognition. Scand J Immunol. 2007