Thesis of Ph.D. dissertation

Mechanism of tumor cell derived galectin-1 induced T cell apoptosis

Ferenc Kovács-Sólyom

supervisor: Éva Monostori Ph.D., D.Sc.

HAS, Biological Research Center of Szeged, Institute of Genetics
Lymphocyte Signal Transduction Laboratory

Introduction

Galectin-1 (Gal-1) is the first discovered, well characterized member of the galectin family. It can compose homodimer and has carbohydrate binding domain which recognizes glycosylated residues of proteins. Several different functions of Gal-1 were described. Its role in the development of olfactory neurons, axons was showed in experiments with mice. Gal-1 modifies the immune response by activation of Th2 cells which change the cytokine profile, so it reduces the inflammatory processes. *In vivo* Gal-1 is involved in induction of T and B cell apoptosis as well as it was identified as an effector molecule of regulatory T cells which initiate the programmed cell death of activated T cells during the elimination of immune response. The protein is important in maintaining of immune privilege developed by tumor cells. Gal-1 is able to bind to glycosylated residues of extracellular matrix proteins, so it influences adhesion processes. By means of this property, it can help migration of tumor cells and penetration through the blood vessel endothelium hereby forming metastasis. In normal, healthy organism the protein has precision controlling role, its relevance can be observed under pathological conditions. Lack of Gal-1 or its functional deficiency results in decreased immune modulator effect therefore chronic inflammation could be developed for example in case of rheumatoid arthritis or psoriasis. Increased activity of the protein is dangerous in tumor diseases because immune regulation could be stronger, progression of the tumor could be faster. The cells inside the growing tumor are under hypoxic conditions. These cells initiate formation of new blood vessels (angiogenesis) for surviving. Gal-1 becomes activated in hypoxia and induces production of angiogenesis stimulating factors, hence it contributes to angiogenesis.

Based on these findings we can say that Gal-1 is a very important, central molecule of tumor cells, it would be a good target for different tumor immunotherapy.

It is strongly suggested that *in vivo* Gal-1 can develop its functions in solid phase (cell membrane or extracellular matrix) bound form since it binds glycoconjugates with high affinity that can be found in the environment of the cells.

Aims

Most of the literature data about Gal-1 induced T cell apoptosis are known from different studies where soluble recombinant protein was used during the experiments. The aim of my work was to investigate the T cell apoptosis inducing ability of cell membrane bound Gal-1 and examine the molecular mechanism of the programmed cell death.
Following questions and tasks were defined:

1.) developing an *in vitro* model system where the cell-cell interaction and apoptosis could be analyzed
2.) to investigate the T cell apoptosis induced by tumor cells in cell culture
3.) to describe whether tumor cell derived, membrane-bound Gal-1 is involved in triggering T cell apoptosis
4.) to investigate the mechanism of T cell apoptosis induced by tumor cell derived Gal-1, and to compare this process to the main steps of soluble recombinant Gal-1 provoked T cell death

Methods

The presence of tumor cell produced Gal-1 on the cell membrane was detected by flow cytometry using anti-Gal-1 antibody produced in our laboratory. Measurements were evaluated by using CellQuest^{TM} software.
Cell-cell interaction was investigated by microscope, apoptosis was detected by using fluorescent dye-conjugated AnnexinV labeling. Involvement of $p56^{lck}$ and ZAP70 kinases in apoptotic process was proved by enzyme deficient T cell lines. Mitochondrial membrane depolarization was showed by using JC-1 which is mitochondrial membrane potential-dependent dye. This molecule accumulates in the healthy mitochondria and then emits in red color, while it can not enter into the mitochondria with decreased membrane potential therefore only the green monomer form of the dye is visible in the

cytoplasm. Activation of caspase 3 in the T cells during the apoptotic pathway was proved by using an antibody which recognizes only the active, cleaved caspase 3.
Gal-1 production in the cell lines was shown by Western blot.
The Gal-1 non-expressing HeLa cells were transfected with Gal-1 cDNA containing pcDNA3.1 vector. The high amount of Gal-1 producing U87 glioma cells were transfected with pSUPERIOR-NEO plasmid wich encoded Gal-1 gene silencing siRNA sequence. Both transfections were performed with $Ca_3(PO4)_2$ mediated method. Stable clones were selected by neomycin resistance.

Eredmények

A special *in vitro* model system was developed in which we could investigate the interaction of tumor cells and T cells. The adherent glioma or melanoma cells were grown onto glass coverslip then activated T cells or Jurkat leukemic T cells which were used as model T cells were added and incubated in the same culture (co-culture). Previously the nuclei of T cells were labeled by Hoechst33342 dye for correct distinguishing. The staining did not influence the viability of the cells. Molecular mechanism of the T cell or Jurkat apoptosis was investigated in this system. We obtained the following results:

1.) the examined Gal-1 expressing melanoma and glioma cells induced apoptosis of T cells in co-culture system in contrast to Gal-1 non-producing HeLa human cervix carcinoma cells
2.) Gal-1 non-producing HeLa cells did not induce T cell apoptosis however they bound the exogenously added human recombinant Gal-1 on their cell surface. The elevated Gal-1 concentration resulted in more membrane bound protein and these HeLa cells were able to trigger T cell apoptosis, the ratio of dead T cells showed a good correlation with Gal-1 concentration
3.) stable clones of Gal-1 cDNA transfected HeLa cells were selected, expressing different levels of Gal-1 protein. The percentage of T cell death induced by these cells was directly proportional to the amount of produced transgenic Gal-1. In

contrast the control HeLa cells which were transfected with empty plasmid did not induce apoptosis of T cells

4.) although U87 glioblastoma cells produced large quantity of Gal-1 when this protein was removed from cell surface by thiodigalactoside (minimal ligand of the protein) the T cell apoptosis inducing ability was reduced

5.) Gal-1 gene was silenced in U87 glioblastoma cells by siRNA technique (success was checked by flow cytometry and Western blot), decreased level of produced Gal-1 resulted in diminished ratio of caused T cell death

6.) since programmed death of T cells occured only in the presence of Gal-1 in co-culture system we can conclude that Gal-1 was the main triggering factor of tumor cell induced T cell apoptosis

7.) during our experiments which aimed to describe the mechanism of T cell death it was observed that concentrated supernatant of Gal-1 producing cell lines did not induce T cell apoptosis

8.) when tumor cells and T cells were separated by a microporous membrane which inhibited the physical interaction of the two cell types but flowing of soluble factors was allowed, T cell apoptosis could not be detected

9.) investigating the direct cell-cell interaction observed during co-culture experiments by confocal microscopy it was visible that Gal-1 protein translocated from tumor cell to the T cells

10.) it is showed by these results that induction of T cell apoptosis required direct cell-cell interaction between tumor and T cells when Gal-1 translocated from tumor cell to the T cells

11.) $p56^{lck}$ and ZAP70 kinase deficient T cells were resistant to tumor cell induced cell death in co-culture system consequently these enzymes were involved in tumor cell derived Gal-1 triggered apoptosis

12.) one of the most characteristic marker of the apoptosis is the translocation of phosphatidil-serin from intracellular side of the cell membrane to the extracellular surface, we detected this phenomenon by labeling with flurescent dye-conjugated AnnexinV

13.) mitochondrial membrane depolarization was observed in T cells co-cultured with Gal-1 expressing tumor cells by using a special, mitochondrial membrane potential-dependent dye which indicates decreasing of membrane potential by color changing

14.) activation of caspase 3 was detected in apoptotic T cells using an antibody which recognize only the cleaved, active enzyme

15.) accordingly, molecular mechanism of tumor cell-derived Gal-1 induced T cell apoptosis corresponded with main steps of recombinant Gal-1 caused programmed T cell death which have published earlier by our group

Based on our findings we can conclude that Gal-1 producing tumor cells induce apoptosis of co-cultured T cells in contrast to Gal-1 non-expressing tumor cells. Direct cell-cell interaction is required for apoptosis induction, where Gal-1 translocates from tumor cell to T cell. Gal-1 produced by tumor cells triggers T cell apoptosis via similar molecular pathway as we described before in case of recombinant Gal-1.

Publications

Papers

Ferenc Kovács-Sólyom, Andrea Blaskó, Roberta Fajka-Boja, Róbert L. Katona, Lea Végh, Julianna Novák, Gábor János Szebeni, László Krenács, Ferenc Uher, Vilmos Tubak, Robert Kiss, Éva Monostori:
Mechanism of tumor cell-induced T-cell apoptosis mediated by galectin-1. *Immunology Letters* in press Impact factor (IF): 2.86

Gabriela Ion, Roberta Fajka-Boja, **Ferenc Kovács**, Gábor Szebeni, Imre Gombos, Ágnes Czibula, János Matkó, Éva Monostori:
Acid sphingomyelinase mediated release of ceramide is essential to trigger the mitochondrial pathway of apoptosis by galectin-1. *Cellular Signaling (2006)* IF: 4.89

Presentations

Kovács-Sólyom Ferenc, Blaskó Andrea, Katona Róbert, Szebeni Gábor János, Krenács László, Végh Lea, Fajka-Boja Roberta, Tubak Vilmos, Robert Kiss, Monostori Éva: A galektin-1, mint legfőbb effektor molekula az U87 glioblasztóma által indukált T sejt apoptózisban
Magyar Immunológiai Társaság XXXVII. Vándorgyűlése, Budapest, 2008. 10. 29-31.

Ferenc Kovács-Sólyom, Andrea Blaskó, Gábor János Szebeni, Vilmos Tubak, László Krenács, Roberta Fajka-Boja, Lea Végh, Éva Monostori:
The role of galectin-1 in the war of tumor cells against T cells
Straub days, Szeged, 2007.11.28-30.

Kovács Ferenc, Tubak Vilmos, Monostori Éva:
Tumorsejtek által termelt galektin-1 T sejtekre gyakorolt citotoxikus hatása
VII.Magyar Genetikai Kongresszus és XIV.Sejt-és Fejlődésbiológiai Napok
Balatonfüred, 2007.04.15-17.

Posters

Kovács-Sólyom Ferenc, Blaskó Andrea, Katona Róbert, Szebeni Gábor János, Krenács László, Végh Lea, Fajka-Boja Roberta, Tubak Vilmos, Robert Kiss, Monostori Éva:
A galektin-1, mint legfőbb effektor molekula az U87 glioblasztóma által indukált T sejt apoptózisban. Kiemelt poszter
38.Membrán-transzport konferencia, Sümeg, 2008.05.20-23.

Kovács Ferenc, Tubak Vilmos, Monostori Éva:
Rákos sejtek által termelt galektin-1 T sejtekre gyakorolt citotoxikus hatása
36.Membrán-transzport konferencia, Sümeg, 2006.05.23-26.

Kovács Ferenc, Monostori Éva:
A galektin-1 tumor ellenes immunszupresszív hatása
Magyar Immunológiai Társaság Vándorgyűlése, Sopron, 2005.10.19-22.

Other publications

Roberta Fajka-Boja, Andrea Blaskó, Ferenc Kovács-Sólyom, Gábor János Szebeni, Gábor K. Tóth and Éva Monostori:
Co-localization of galectin-1 with GM1 ganglioside in the course of its clathrin-and raft-dependent endocytosis. *Cellular and Molecular Life Sciences (2008)* IF: 5.24

Acknowledgement

I would like to express my thanks for my supervisor,

Éva Monostori

and for the members of the Lymphocyte Signal Transduction Laboratory:

Roberta Fajka-Boja, Andrea Blaskó, János Szebeni Gábor, Julianna Novák,
Lea Végh, Mrs. András Gercsó

Thank you
Vilmos Tubak, Róbert L. Katona, Péter Blazsó, László Krenács,
Edit Kotogány, Ayaydin Ferhan, Zsuzsá Kószó, Mrs. Sándor Tóth.

Thank you for cell lines
Robert Kiss, Arthur Weiss and Robert T. Abraham.