Mesenchymal stem cell-derived galectin-1 is a determining factor in the pro-angiogenic function of adult tissue stem cells

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Ph.D. thesis

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Introduction

Vascular system plays crucial role in the transport of nutrients to the tissues and removal of the catabolic products from the tissues, delivery of hormones and other biologically active molecules such as cytokines ensuring the hormonal communication between the organs and the rapid immune response. During the body growth, the temporal hypoxic condition triggers production of pro-angiogenic factors which in turn stimulate the sprouting of pre-existing vessels followed by the development of tight strings by the contact of endothelial cells (EC). Endothelial cells produce specific factors which recruit periendothelial cells and support the stabilization of the new vessels.

Several *in vitro* model systems exist to study angiogenesis, one among these is the pre-vascular structure assay. In this model ECs and periendothelial cells are co-cultured which results in the formation of specific cell-cell alignments. The extension of these structures are analyzed in the presence and absence of pro- and anti-angiogenic factors.

During the postnatal life period the physiological vascularization is tightly regulated and limited to tissue growth and tissue regeneration while intensive angiogenesis occurs in the developing and growing solid tumors. In solid tumors the rapid cell proliferation causes hypoxic tissue area due to nutrient and oxygen deprivation. As a response the tumor cells and the tumor associated stroma cells produce uncontrolled amount of pro-angiogenic factors resulting in vessels largely different from those of the physiological ones. Other stromal elements than vessels such as tumor-associated fibroblasts (TAF) and periendothelial cells including pericytes and vascular smooth muscle cells are required for effective tumor growth. The origin of these cells is poorly known, one possible source could be the bone marrow mesenchymal stem cells (MSCs). MSCs are adult multiponent tissue stem or stromal cells occurring in low amount in all adult tissue. MSCs are responsible the renewal of the tissue connective tissues under physiological conditions. Upon malignant conditions MSCs may be mobilized and migrate into the tumor tissue where they support tumor development. Moreover they may contribute to tumor immunoprivilege and stimulate tumor angiogenesis via supporting heterotypic cellular interactions.

Beside the large number of tumor promoting bioactive molecules MSCs produce abundant galectin-1 (Gal-1), a β-galactoside binding lectin. Gal-1 acts as a powerful anti-inflammatory, immunosuppressive factor therefore it is implicated in the maintenance of the immunohomeostasis. Hence Gal-1 is highly expressed in immunoprivileged sites contributing to the physiological immunosuppression in these tissues. Not surprisingly Gal-1 is also highly expressed in solid tumors supporting the tumor growth with various strategies: promotes

tumor cell proliferation, and migration, contributes to the immunoprivilege of tumors by triggering apoptosis of tumor specific T lymphocytes and enhances angiogenesis by increasing EC proliferation and survival.

Since Gal-1 endorses to tumor progression on various ways it serves as a potential target for tumor therapy. Inhibiting tumor Gal-1 may stimulate tumor specific immune response, may reduce tumor angiogenesis and metastasis.

Aims

Major question: How does MSC-derived Gal-1 regulate tumor growth? To get an answer we investigate the following points:

1.) How do MSCs and ECs affect proliferation of each other in co-culture and does MSC-derived Gal-1 participate in regulation of proliferation?

2.) Does formation of pre-vascular structure depend on intimate cell contact between MSCs and ECs or soluble factors? Does MSC-derived Gal-1 play a role in this process?

3.) Does MSC-derived Gal-1 have function in tumor-promoting effect of MCSs? What role MSC-derived Gal-1 plays in the immunosuppressive function and pro-angiogenic effect of MSCs?

Methods

Cell cultures were carried out in a humidified CO_2 thermostat, Pre-vascular structure assay occurred in co-culture of MSCs and endothelial cells for 48 hours and the results were evaluated by measuring tube lenghts using CellR Imaging softver. Effect of soluble factors were studied either in transwell system or co-culture conditioned tissue culture medium. Growth of heterotypic cell cultures were analyzed by labeling cells with CFSE fluorescent dye, measured with flow cytometry and evaluated using CellQuest softver.

In vivo effect of MSCs to tumor development was studied in two mouse model systems: Either wild type or Gal-1 knockout MSCs were co-injected orthotopically with mouse breast cancer cells or were transplanted intravenously into tumor bearing animals. To determine whether MSC-derived Gal-1 acts on the tumor specific immune response, XSCID mice were co-transplanted with Gal-1 expressing and Gal-1 deficient MSCs and mouse

melanoma. Tumor growth was followed by measuring tumor volume with a precision caliper every second day and microvessel density was evaluated on histochemical samples.

Results

- **Adaptation of pre-vascular test to study MSCs' pro-angiogenic function *in vitro***

  - Co-culture of MSCs and ECs had an impact of each other's proliferation and viability. Although co-cultivating MSC^{wt} and ECs did not have any effect on growing of ECs, $MSC^{Gal\text{-}1\text{-}/\text{-}}$ inhibited proliferation of ECs on a dose dependent fashion. In contrast to this finding ECs had moderate but significant impact on growing of MSC^{wt} and $MSC^{Gal\text{-}1\text{-}/\text{-}}$. Moreover, viability of ECs was reduced in the presence of Gal-1 knockout MSCs.

  - MSC-derived Gal-1 influenced length of pre-vascular cell clusters in EC-MSC co-culture. More structures evolved in the presence of MSC^{wt} than in contact with $MSC^{Gal\text{-}1\text{-}/\text{-}}$ and the structures developed between ratios of ECs: MSC^{wt} = 5:1 and 1:1 while in the ratio 1:5 only short clusters were observed. In case of inhibiting Gal-1 by thiodigalactoside less pre-vascular structures formed in the MSC^{wt} and ECs co-culture than in the control samples, which indicated the extracellular function of Gal-1.

  - Direct contact between endothelial and mesenchymal stem cells was essential to the formation of MSC/EC network, a result which was proved by two methods: 1) in Transwell system, where MSCs grew in an insert being physically separated from ECs and 2) using a combination of MSC-derived extracellular matrix and single cell culture or co-culture derived conditioned medium. Neither of them supported the growing of structures.

- **Intravenously administered MSC supported the growth of established tumor depending on Gal-1 expression**

  - Mice were orthotopically injected with breast carcinoma cells and after development of palpable tumor they were treated with MSC^{wt} or $MSC^{Gal\text{-}1\text{-}/\text{-}}$ intravenously. Gal-1

expressing MSCs promoted growth of carcinoma similar to that model in which MSCs and breast tumor cells were previously mixed and co-transplanted into breast tissue.

- The growth promoting effect of MSCs was dependent on Gal-1 expression, a powerful immunosuppressive protein. However, when comparing melanoma growth co-injected with MSC^{wt} or $MSC^{Gal\text{-}1\text{-}/\text{-}}$ in X-SCID immunodeficient and not immuno-compromised wild type mice no difference was found indicating that MSC-derived Gal-1 did not express its immunosuppressive activity in tumorogenic conditions. On the other hand, histochemical analysis of breast tumor sections indicated a role of Gal-1 in tumor vascularization since significantly higher tumor vascularization was evolved in the presence of MSC^{wt} than in $MSC^{Gal\text{-}1\text{-}/\text{-}}$.

Novel findings:

- **Pre-vascular structure assay, one of the *in vitro* angiogenesis models, has been adapted and improved to become suitable for studying the regulatory effect of MSC during angiogenesis. Using this model the following conclusions have been stated:**
  - ECs significantly stimulate growth of MSC^{wt} as well as $MSC^{Gal\text{-}1\text{-}/\text{-}}$
  - establishment of direct contact between ECs and MSCs is essential to the formation of pre-vascular network
  - the length of the pre-vascular structures greatly depends on the expression of Gal-1 in MSCs and on the ratio of EC to MSC in co-cultures.

- **The role of MSC-derived Gal-1 in regulation of tumor growth by the MSCs was confirmed in tumor bearing animals**
  - Intravenously administered MSCs support developement of breast carcinoma depending on Gal-1 expression in established tumor bearing mice similarly to the model (see GJ Szebeni's thesis) in which MSCs and carcinoma cells are co-transplanted.
  - Tumor-promoting function of MSC-derived Gal-1 is not due to its immunosuppressive feature rather because of its pro-angiogenic function.

Summary

The results of pre-vascular structure assay suggest, that mesenchymal stem cell-derived galectin-1 plays important role in promotion of angiogenesis. The *in vitro* findings were strongly supported by the *in vivo* tumor model systems.

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Publications closely related to this work

Szebeni GJ*, **Kriston-Pál É***, Blazsó P, Katona RL, Novák J, Szabó E, Czibula Á, Fajka-Boja R, Hegyi B, Uher F, Krenács L, Joó G, Monostori É. ***Identification of galectin-1 as a critical factor in function of mouse mesenchymal stromal cell-mediated tumor promotion***. PLoS One. 2012; 7(7):e41372. IF(2012): 3.73

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Novák J, **Kriston-Pál É**, Czibula Á, Deák M, Kovács L, Monostori É, Fajka-Boja R. ***GM1 controlled lateral segregation of tyrosine kinase Lck predispose T-cells to cell-derived galectin-1-induced apoptosis***. Molecular Immunology 2014; 57(2):302-9. IF(2012): 2.64

Publications loosely related to this work

Hegedüs Z, Wéber E, **Kriston-Pál É**, Makra I, Czibula Á, Monostori É, Martinek TA. ***Foldameric α/β-peptide analogs of the β-sheet-forming antiangiogenic anginex: structure and bioactivity***. Journal of American Chemical Society 2013; 135(44):16578-84. IF(2012): 10.67

Hegedüs Z, Wéber E, Végh L, Váczi B, Tubak V, **Kriston-Pál É**, Kele Z, Martinek TA. ***Two-stage interaction of the tumor nursing galectin-1 with the antiangiogenic peptide anginex***. Journal of Thermal Analysis and Calorimetry 2014; DOI 10.1007/s10973-014-4031-9 IF(2012): 1.9

Oral presentations

1. Kriston-Pál Éva, Szebeni Gábor János, Novák Julianna, Czibula Ágnes, Szabó Enikő, Fajka-Boja Roberta, Uher Ferenc, Krenács László, Monostori Éva. ***A mesenchymalis őssejtek által termelt galektin-1 az érképződés serkentésével befolyásolja a tumorfejlődést.*** Magyar Immunológiai Társaság 41. Vándorgyűlése, Debrecen, 2012.10.17-19.

2. Kriston-Pál Éva
   A galektin-1 fehérje szerepe a kapilláris-szerű struktúrák kialakulásában mezenhimális őssejt és endotélsejt ko-kultúrában. Szegedi Biológus Doktorandusz Konferencia, Szeged, 2012.05.17-18.

3. Kriston-Pál Éva
   Csontvelői eredetű mezenhimális őssejtek (MSC) és az MSC eredetű galektin-1 hatása fiziológiás és patológiás érképződésre. Sófi József Ösztöndíj konferencia, Szeged, 2011.

Conference posters

1. Éva Kriston-Pál*, Gábor János Szebeni*, Julianna Novák, Roberta Fajka-Boja, Enikő Szabó, Ágnes Czibula, Ferenc Uher, Éva *Monostori*. ***Identification of galectin-1 as a critical factor in function of mouse mesenchymal stem cell-mediated tumor promotion***. Stem cells in cancer and regenerative medicine, Heidelberg, Germany, 2012.08.29.

2. Kriston-Pál Éva, Fajka-Boja Roberta, Czibula Ágnes, Szebeni Gábor János, Uher Ferenc, Monostori Éva. ***Mezenhimális őssejt-eredetű galektin-1 hatása az in vitro érdifferenciációra***. Magyar Immunológiai Társaság 40. Vándorgyűlése, Szeged, 2011.10.12-14.

3. Éva Kriston-Pál, Roberta Fajka-Boja, Julianna Novák, Gábor János Szebeni, Ferenc Uher, Éva Monostori. ***Galectin-1 is required to the formation of capillary-like structures in mesenchymal stem cell and endothelial cell co-culture.*** Impulse Szimpózium, Visegrád, 2011.09.3-6.