The International Journal of Biochemistry & Cell Biology 86 (2017) 26–31

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

The protective activity of mesothelial cells against peritoneal growth of gastrointestinal tumors: The role of soluble ICAM-1

Justyna Mikuła-Pietrasik[a], Paweł Uruski[a], Małgorzata Kucińska[b], Andrzej Tykarski[a], Krzysztof Książek[a,*]

[a] Department of Hypertensiology, Angiology and Internal Medicine, Poznań University of Medical Sciences, Długa 1/2 Str., 61-848 Poznań, Poland
[b] Department of Toxicology, Poznań University of Medical Sciences, Dojazd 30 Str., 60-631 Poznań, Poland

ARTICLE INFO

Article history:
Received 20 November 2016
Received in revised form 12 February 2017
Accepted 13 March 2017
Available online 18 March 2017

Keywords:
Adhesion molecules
Colorectal cancer
Cancer metastasis
Mesothelial cells
Pancreatic cancer

ABSTRACT

In this project we examined how the presence of human peritoneal mesothelial cells (HPMCs) modifies (supports or inhibits) colorectal and pancreatic cancer cell progression in mice peritoneal cavity. Experiments were performed using primary, omentum-derived HPMCs, commercially available colorectal (SW-480) and pancreatic (PSN-1) cancer cells, and immunocompromised SCID mice. Tumor growth within the peritoneal cavity was monitored using bioluminescence. Adhesion of the cancer cells to HPMCs was examined using a fluorescence-based method, while the incidence of apoptosis was quantified using flow cytometry. Experiments showed that SW480 and PSN-1 cells formed tumors *in vivo* at higher efficiency when they were injected alone than in the presence of HPMCs. *In vitro* investigations confirmed that firm adhesion of SW480 and PSN-1 cells to HPMCs is mediated by interactions between ICAM-1 and CD43. They also revealed that IL-6 and TNFα up-regulate the expression of cell-bound ICAM-1 and the secretion of soluble ICAM-1 (sICAM-1). The basal release of sICAM-1 by HPMCs positively correlated with the expression of the cell-bound molecule. sICAM-1 inhibited dose-dependently the adhesion of SW480 and PSN-1 cells to HPMCs. Cancer cells that did not adhere to HPMCs displayed increased activity of caspase-3 and -9, increased incidence of apoptosis, and an inability to re-adhesion, as compared with their intact counterparts not exposed to sICAM-1. Our findings indicate that under certain conditions HPMCs are capable of inhibiting growth of gastrointestinal tumors in a mechanism involving the anti-adhesive capabilities of sICAM-1.

1. Introduction

Peritoneal cavity is a common place for ovarian, colorectal and pancreatic cancer metastasis (Lengyel, 2010; Lu et al., 2010). There is a paradigm that intraperitoneal spread of cancer is facilitated by a permissive microenvironment generated by normal cells, especially mesothelium (HPMCs) and fibroblasts (HPFBs) (Sandoval et al., 2013). Cancer-supporting activity of the normal peritoneal cells has been explored most deeply for ovarian tumors. It has been found that the very early stages of ovarian cancer cell invasion are promoted primarily by the HPMCs'-derived fibronectin (Kenny et al., 2014), whereas further steps of a metastasis formation are fueled by several molecular interactions between cancer cells and HPMCs/HPFBs (Kenny et al., 2007; Mikula-Pietrasik et al., 2014b; Ren et al., 2006).

As per gastrointestinal cancers, their interactions with HPMCs are far less clear. What is known is that adhesion of colorectal and pancreatic cancer cells to HPMCs is mediated by interactions between intercellular adhesion molecule 1 (ICAM-1) on the surface of HPMCs and CD43 on cancer cells (Ksiazek et al., 2010). In addition, colorectal tumors (Mikula-Pietrasik et al., 2015; Ranieri et al., 2013), similarly to ovarian tumors (Mikula-Pietrasik et al., 2016), but conversely to pancreatic ones (Mikula-Pietrasik et al., 2015), progress at higher efficiency when they are accompanied by senescent HPMCs.

In this report we show, using both *in vitro* experiments and immunocompromised mice, a contradictory finding that the presence of HPMCs may protect the peritoneal cavity against the dissemination of colorectal and pancreatic tumors. In this context we provide evidence that from a mechanistic point of view this unexpected result is elicited by the anti-adhesive capabilities of soluble form of ICAM-1 (sICAM-1).

* Corresponding author.
E-mail addresses: jmikula@ump.edu.pl (J. Mikuła-Pietrasik), puruski@ump.edu.pl (P. Uruski), kucinska@ump.edu.pl (M. Kucińska), tykarski@o2.pl (A. Tykarski), kksiazek@ump.edu.pl (K. Książek).

http://dx.doi.org/10.1016/j.biocel.2017.03.013
1357-2725/

2. Materials and methods

2.1. Chemicals

Unless otherwise stated, all reagents and plastics were from Sigma-Aldrich Corp. (St. Louis, MO, USA). Recombinant proteins and specific neutralizing antibodies against ICAM-1 were from R&D Systems (Abingdon, UK). Neutralizing antibody against CD43 was from Invitrogen (Carlsbad, CA, USA). Appropriate isotype-matched IgG controls were obtained from R&D Systems.

2.2. Cell cultures

HPMCs were isolated from fragments of omentum by enzymatic (trypsin-EDTA) disaggregation in a shaking water bath for 20 min. The tissues were obtained from 34 patients undergoing elective abdominal surgery. The age of the donors ranged from 19 to 42 years. HPMCs were propagated in M199 medium with 10% fetal bovine serum (FBS) and L-glutamine (2 mM). Experiments were performed on cells from the 2nd passage. The experiments were approved by institutional ethics committee (consent numbers: 187/14 and 543/14).

Colorectal cancer cell line SW480 was purchased from the American Type Culture Collection (Rockville, MD, USA) and pancreatic cancer cells, PSN-1, was obtained from the European Collection of Cell Cultures (Porton Down, UK). The cancer cells were maintained in RPMI-1640 with 10% FBS.

2.3. Adhesion assay

Adhesion of cancer cells to HPMCs was performed using a fluorescence method with calcein-AM, as described in (Ksiazek et al., 2010). In some experiments the cancer cell adhesion was examined in the presence of exogenous, recombinant sICAM-1 (0–3 ng/ml) or in the presence of autologous HPMC-derived medium containing endogenous sICAM-1 (1 ng/ml) upon its pre-treatment (for 6 h) with the anti-ICAM-1 antibody (20 μg/ml). Another time, adhesion of cancer cells was tested upon their pre-incubation with anti-CD43 antibody (10 μg/ml for 6 h), the pre-incubation of HPMCs with anti-ICAM-1 antibody (20 μg/ml for 6 h), or the simultaneous pre-incubation of the cancer cells and the HPMCs with both types of antibodies.

2.4. Immunoassays

Cell-bound ICAM-1 was assessed in HPMCs with an immunoassay using specific mouse anti-human ICAM-1 antibody, as described in (Ksiazek et al., 2010). Concentration of sICAM-1 was determined with appropriate DuoSet® Immunoassay Development kit (R&D Systems, Abingdon, UK), according to manufacturer's instructions. In some experiments, the expression of cell-bound ICAM-1 and the secretion of sICAM-1 were evaluated upon the exposure of HPMCs to exogenous IL-6 and TNFα (0.1–10 ng/ml for 6 h).

2.5. Analysis of apoptosis

The activity of caspases was quantified in cell lysates using specific colorimetric kits (Abcam, Cambridge, UK), according to the manufacturer's instructions. The incidence of apoptosis was examined according to the presence of subG$_1$ fraction in cells stained with propidium iodide and analysed using a FACSCalibur™ flow cytometer with Cell-Quest™ software (Becton-Dickinson, Plymouth, UK).

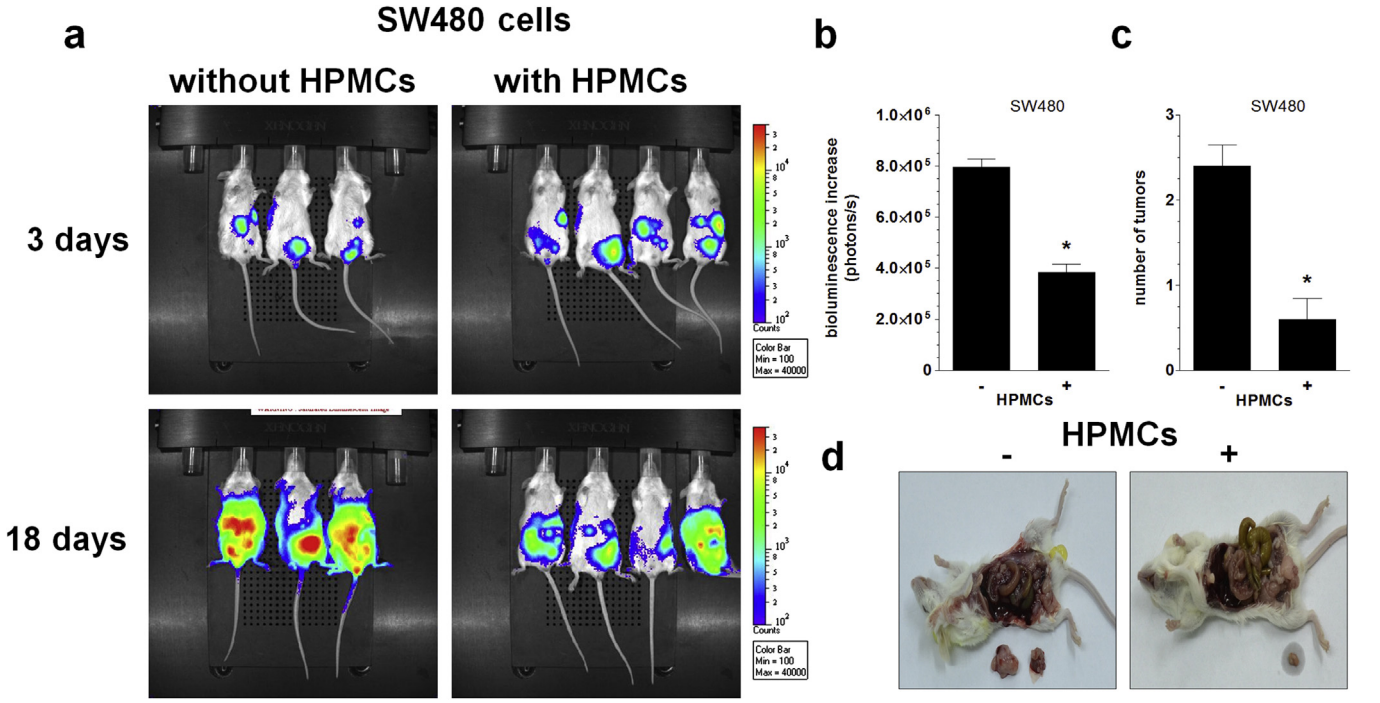

Fig. 1. Bioluminescence-based analysis of the intraperitoneal development of colorectal tumors in mice injected with cancer cells alone or in combination with HPMCs. Representative images showing bioluminescence intensity recorded 3 and 18 days after cell injection **(a)**. The dynamics of the xenograft development estimated according to the difference between the highest bioluminescence intensity recorded throughout the experiment and the initial value recorded 3 days after cell injection **(b)**. Differences in the average number of tumors that developed in the absence or presence of HPMCs throughout the experiment **(c)**. Pictures of representative mice and tumors excised from their peritoneal cavity at the end of experiment **(d)**. The asterisks indicate a significant difference as compared with xenografts established without the HPMCs. Experiments were performed on 6 animals per group.