Pathol. Oncol. Res. (2015) 21:827–830
DOI 10.1007/s12253-014-9812-8

COMMUNICATION

Evaluation of Antitumor Activity of Platelet Microbicidal Protein on the Model of Transplanted Breast Cancer in CBRB-Rb(8.17) 1Iem Mice

Iuri B. Ivanov · Viktor A. Gritsenko · Sergey A. Miroshnikov

Received: 3 April 2014 / Accepted: 4 June 2014 / Published online: 30 November 2014

Abstract Breast cancer is the most common women's cancer in the world. There is considerable current interest in developing anticancer agents with a new mode of action because of the development of resistance by cancer cells towards current anticancer drugs. Mamalian cells have been shown to contain small, cationic, microbicidal peptides. Antimicrobial peptides have drawn attention as a promising alternative to current antitumor agents. Such peptides have been isolated both from animal and human platelets and have been termed platelets microbicidal proteins (PMP). The aim of this work was to study antitumor activity of PMP in vivo on the model of mouse breast cancer in comparison with antitumor hexapeptide Arg-alpha-Asp-Lys-Val-Tyr-Arg (Immunofan). We demonstrated that the tumors treated with PMP were significant smaller than the control groups ($P<0.05$). In experiments in vivo using CBRB-Rb(8.17)1Iem mice with transplanted tumors PMP inhibited tumor growth during the treatments and after its discontinuation. These findings indicate that PMP can exert antitumor effects. Therefore, PMP may be used for the development of therapy for the intervention of breast cancer.

Keywords Platelet microbicidal protein · Antitumor activity · Breast cancer · Mouse model

I. B. Ivanov (✉) · V. A. Gritsenko
Institute of Cellular and Intracellular Symbiosis, Russian Academy of Sciences, Orenburg, Russia
e-mail: ubi2010@rambler.ru

S. A. Miroshnikov
All-Russian Institute of Meat Cattle Breeding, Russian Academy of Agricultural Sciences, Orenburg, Russia

Introduction

Breast cancer (BC) is the most common women's cancer in the world. The effective therapies that would not only reduce the high mortality rate associated with the disease, but also improve the quality of life patients with breast cancer are still searching for [1].

Depending on indications there are few options: surgical treatment, radiotherapy, hormonotherapy, chemotherapy and targeted therapy. Although targeted therapies have improved patient survival for advanced BC, these tumors frequently relapse due to drug resistance mechanisms [2].

Therefore, new strategies are urgently needed, and the challenge for the future will most likely be the development of individualized therapies that specifically target each patient's tumor [3]. Thus, there is considerable current interest in developing anticancer agents with a new mode of action because of the development of resistance by cancer cells towards current anticancer drugs. The idea to use therapy peptides has gaining increased popularity to treat cancerous tumors. Antimicrobial peptides (AMP) have drawn attention as a promising alternative to current antitumor agents [4].

Shamova and colleagues [5] investigated effects of two structurally different cationic antimicrobial peptides of cathelicidin family, porcine protegrin 1 and caprine bactenecin 5 on selected tumor and normal mammalian cells in vitro. They have shown that while protegrin 1 exerts distinct and fast cytotoxic effects on most of used tumor cells being slightly less toxic for nontransformed host cell, the proline-rich bactenecin 5 is much less cytotoxic for all the cells tested.

Blood platelets from patients with cancer (before or after the surgery) exhibit a variety of qualitative abnormalities: aggregation (induced by thrombin) of blood platelets from patients with breast cancer before the surgery, after the surgery, and after various phases of the chemotherapy differs from aggregation of platelets obtained from healthy volunteers

[6, 7]. On the other hand, platelet mimicry of certain cancer types (e.g. breast, prostate, colorectal and urogenital cancers) is an important factor in their hematogenous dissemination and provides an attractive therapeutic target [8].

Moreover, platelets are essential for the innate immune response and combat infection (viruses, bacteria, micro-organisms). They help maintain and modulate inflammation and are a major source of pro-inflammatory molecules (e.g. P-selectin, tissue factor, CD40L, metalloproteinases). As well as promoting coagulation, they are active in fibrinolysis, wound healing, angiogenesis etc. [6, 9].

Recently we claimed in the Patent of Russian Federation No. 2419451 antitumor effects of platelet microbicidal protein (PMP) [10].

Thus, in this study we examined antitumor activity of PMP on the model of transplanted breast cancer in CBRB-Rb(8.17)1Iem mice.

Materials and Methods

Thirty CBRB-Rb(8.17)1Iem male mice (hereafter called CBRB) from the laboratory animal department at Shemyakin-Ovchinnikov Institute of Organic Biochemistry were used in this work and divided into three equal groups. Their average age was 13.2±3.2 months and the average weight was 25.9±2.6 g. At day 0, 1.3×10^7 mammary carcinoma cells ErbB2 taken from a fast growing carcinoma were transplanted s.c. to mice withers according to Vodovozova and colleagues [11]. Mice were inspected each day for survival and health monitoring, and once a week for tumor size measurement.

The individual tumor growth was expressed as the mean tumor diameter dynamics using the formula $D=(a+b+h)/3$; where D is the mean tumor diameter, a is the maximal length, b is the maximal width, and the h is the average height of the regularly shaped tumor as this approach was used to visualize the linear tumor growth phase [11]. As described earlier by Maas et al. [12], the mean tumor diameter is an adequate criterion to present tumor size differences among experimental groups.

PMP from human platelets was prepared and standardized as described previously [13]. PMP preparations had bactericidal activity against Bacillus subtilis ATTC 6633 at 2.08± 0.5 μg/ml.

At days 2, 4, 6, 8, 10 the mice were treated peritumorally with PMP suspended in 0·2 ml 0·9 % NaCl in final concentration 1 μg/ml. Control tumor-bearing mice in first group injected peritumorally with 0·2 ml 0·9 % NaCl, in second control group mice were treated with 0.2 ml the original hexapeptide Arg-alpha-Asp-Lys-Val-Tyr-Arg with antitumor activity [14] (Immunofan, Bionox Ltd, Moscow) in final concentration 1 μg/ml.

All experiments were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (U.S. Department of Health and Human Services, National Institute of Health).

The significance of differences between groups was determined with the parametric Student's t-test.

Results and Discussion

As shown (Table 1), the tumors treated with PMP were significant smaller than the control group ($P<0.05$). Moreover, the maximal therapeutic effect of PMP was detected at 16th day after treatment (26th day of experiment). On the other hand we didn't registered significantly differences between tumor diameter in control group and group treated with Immunofan. The average diameter of tumors were also lower in the PMP group compared to the Immunofan and control mice ($P<0.05$). PMP injections didn't affect on weight of recipients in comparison to control group.

In the last decade intensive research has been conducted to determine the role of innate immunity host defense peptides (also termed antimicrobial peptides) in the killing of prokaryotic and eukaryotic cells. Many antimicrobial peptides damage the cellular membrane as part of their killing mechanism. However, it is not clear what makes cancer cells more susceptible to some of these peptides, and what the molecular mechanisms underlying these activities are. To be clinically used, these peptides need to combine high and specific anticancer

Table 1 Tumor growth rate of CBRB-Rb(8.17)1Iem male mice with s.c. mammary carcinomas during the peritumorally treatment

Therapeutic agents	Days post s.c. mammary carcinomas transplantation						
	2	5	10[a]	15	20	25	30
NaCl	0.1±0.03	0.3±0.08	0.5±0.04	0.9±0.08	2.2±0.08	5.1±0.08	8.3±0.09
IF	0.1±0.03	0.2±0.02	0.6±0.05	0.9±0.05	2.1±0.07	5.3±0.07	7.0±0.06*
PMP	0.1±0.03	0.4±0.01	0.4±0.03**	0.4±0.02*,**	0.9±0.04*,**	2.2±0.03*,**	6.1±0.02*,**

* $p<0.05$ in comparison to NaCl
** $p<0.05$ in comparison to IF
[a] Final day of the peritumorally treatment

activity with stability in serum. Although so far very limited, new studies have paved the way for promising anticancer host defense peptides with a new mode of action and with a broad spectrum of anticancer activity [15–17].

Platelets are specialized blood cells that play central roles in physiologic and pathologic processes of hemostasis, inflammation, tumor metastasis, wound healing, and host defense. Activation of platelets is crucial for platelet function that includes a complex interplay of adhesion and signaling molecules [8, 18]. Moreover, platelets and some tumors can facilitate thrombin formation, and since thrombin, either directly or indirectly, may have both growth-promoting and growth-inhibiting effects [18].

Yeaman et al. [19] have shown that human PMPs include known chemokines, such as platelet factor-4 family kinocidins and revealed recurring themes of structure and activity in these multifunctional host defense molecules. Krijgsveld et al. demonstrated that antimicrobial peptides from platelets are derived from CXC-chemokines [20]. And Vandercappellen et al. [21] have shown that CXC chemokines platelet factor-4 (CXCL4/PF-4) and its variant CXCL4L1/PF-4var, recently isolated from thrombin-stimulated platelets, differing from authentic CXCL4/PF-4 in three carboxy-terminally located amino acids, affect tumor development and metastasis by acting as growth factor, by attracting anti-tumoral leukocytes and by inhibiting angiogenesis and tumor growth. Moreover, Struyf and Van Damme [17] described a most potent new angiostatic chemokine, namely a variant of PF-4, designated PF-4var/CXCL4L1. This chemokine inhibits pro-angiogenic tumoral factors, thereby limiting tumor growth and metastasis.

In a recent review by Lippi and Favaloro [22] a role for PF-4 and derivative peptides in inhibiting tumor growth and spread, by suppression of tumor-induced neovascularization in many different types of solid tumors was described. PF4 regulates apoptotic death through activation of distinct signal transduction pathways, inhibits growth factor receptor binding, amplifies the inflammatory response of natural killer cells through regulation of cytokines production, and induces and maintains a nonspecific immune response to cancer cells. These biological evidences paved the way for the development and marketing of novel PF4-based angiostatic agents characterized by reduced toxicity and improved bioavailability, thus raising the possibility of an alternative approach for preventing and treating growth and metastasis of tumors.

Taken together, our results and results of several studies [5, 11, 16, 17, 19, 22] suggest that the identification of novel molecules as therapeutic agents could provide new approaches for molecular-based diagnostic and therapeutic support to treat tumors of different localization as well as other malignancies. However, our results are preliminary and future research based on animal and human models (in vitro and in vivo) is needed to elucidate their in vivo efficacies and toxities and their cooperative utility in clinical practice. Nonetheless, we believe that PMPs should be taken into consideration for use in molecular-based therapeutic approaches for BC treatment.

Conflict of Interest The authors declare that there is no conflict of interests regarding the publication of this article.

References

1. Biersack B, Ahmad A, Sarkar FH, Schobert R (2012) Coinage metal complexes against breast cancer. Curr Med Chem 19:3949–3956
2. Trapé AP, Gonzalez-Angulo AM (2012) Breast cancer and metastasis: on the way toward individualized therapy. Cancer Genomics Proteomics 9:297–310
3. Caffarel MM, Andradas C, Pérez-Gómez E, Guzmán M, Sánchez C (2012) Cannabinoids: a new hope for breast cancer therapy? Cancer Treat Rev 38:911–918
4. Hou L, Zhao X, Wang P, Ning Q, Meng M, Caigang L (2013) Antitumor activity of antimicrobial peptides containing CisoDGRC in CD13 negative breast cancer cells. PLoS ONE 8:e53491. doi:10.1371/journal.pone.0053491
5. Shamova OV, Sakuta GA, Orlov DS, Zenin VV, Shteĭn GI, Kolodkin NI, Afonina IV, Kokriakov VN (2007) Effects of antimicrobial peptides of neutrophils on tumor and normal cells in culture. Tsitologiia 49:1000–1010
6. Kedzierska M, Czernek U, Szydłowska-Pazera K, Potemski P, Piekarski J, Jeziorski A, Olas B (2013) The changes of blood platelet activation in breast cancer patients before surgery, after surgery, and in various phases of the chemotherapy. Platelets 24(6):462–468
7. Tímár J, Tóvári J, Rásó E, Mészáros L, Bereczky B, Lapis K (2005) Platelet-mimicry of cancer cells: epiphenomenon with clinical significance. Oncology 69:185–201
8. Yeaman MR (2010) Platelets in defense against bacterial pathogens. Cell Mol Life Sci 67:525–544
9. Nurden AT (2011) Platelets, inflammation and tissue regeneration. Thromb Haemost 105(Suppl 1):S13–S33
10. Ivanov IB, Miroshnikov SA, Lebedev SV, Notova SV, Sipailova OI, Gritsenko VA (2011) Antitumor medication RU Patent No. 2419451
11. Vodovozova EL, Moiseeva EV, Grechko GK, Gayenko GP, Nifant'ev NE, Bovin NV, Molotkovsky JG (2000) Antitumor activity of cytotoxic liposomes, equipped with selectin ligand SiaLeX, in mouse mammary adenocarcinoma. Eur J Cancer 36:942–949
12. Maas RA, Dullens HFJ, De Jong WH, Den Otter W (1989) Immunotherapy of mice with a large burden of disseminated lymphoma with low-dose interleukin-2. Cancer Res 49:7037–7040
13. Ivanov IB, Gritsenko VA (2009) Comparative activities of cattle and swine platelet microbicidal proteins. Probiotics Antimicrob Proteins 1:148–151
14. Korystov YN, Lebedev VV, Shaposhnikova VV, Ermakova NV, Kublik LN, Chailakhyan LM (2004) The hexapeptide immunofan inhibits the MRP-dependent multidrug resistance of tumor cells. Dokl Biol Sci 395:177–180
15. Mei HF, Jin XB, Zhu JY, Zeng AH, Wu Q, Lu XM, Li XB, Shen J (2012) β-defensin 2 as an adjuvant promotes anti-melanoma immune responses and inhibits the growth of implanted murine melanoma in vivo. PLoS One 7(2):e31328. doi:10.1371/journal.pone.0031328
16. Papo N, Shai Y (2007) Host defense peptides as new weapons in cancer treatment. Cell Mol Life Sci 62:784–790
17. Struyf S, Van Damme J (2007) The role of plasma chemokines in cancer. Verh K Acad Geneeskd Belg 69(3):149–165

18. Coller BS (2012) A brief history of ideas about platelets in health and disease. In: Michelson AD (ed) Platelets. Academic, New York, pp xix–liv
19. Yeaman MR, Yount NY, Waring AJ, Gank KD, Kupferwasser D, Wiese R, Bayer AS, Welch WH (2007) Modular determinants of antimicrobial activity in platelet factor-4 family kinocidins. Biochim Biophys Acta 1768:609–619
20. Krijgsveld J, Zaat SA, Meeldijk J, van Veelen PA, Fang G, Poolman B, Brandt E, Ehlert JE, Kuijpers AJ, Engbers GH, Feijen J, Dankert J (2000) Thrombocidins, microbicidal proteins from human blood platelets, are C-terminal deletion products of CXC chemokines. J Biol Chem 275:20374–20381
21. Vandercappellen J, Van Damme J, Struyf S (2011) The role of the CXC chemokines platelet factor-4 (CXCL4/PF-4) and its variant (CXCL4L1/PF-4var) in inflammation, angiogenesis and cancer. Cytokine Growth Factor Rev 22(1):1–18. doi:10.1016/j.cytogfr.2010.10.011
22. Lippi G, Favaloro EJ (2010) Recombinant platelet factor 4: a therapeutic, anti-neoplastic chimera? Semin Thromb Hemost 36(5):558–569. doi:10.1055/s-0030-1255450