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14. ABSTRACT
During the first year of support, we developed a marked progress toward the main goal of our proposal – understanding the mechanisms of chemokine regulation in prostate cancer. Specifically, we revealed that prostate cancer cell lines and tissues obtained from cancer patients express low or no CXCL14 chemokine protein and mRNA, which might results in low infiltration of the tumor mass by dendritic cells. Importantly, if dendritic cells are not attracted to the prostate cancer tissues, no antitumor immune responses may be generated due to the absence of tumor antigen recognition, processing and presentation. These fundamental findings will now allow us to move forward and investigate the biological significance of these findings and the mechanisms of CXCL14 regulation in tumor cells.

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Introduction

Considerable experimental evidence has recently accumulated that chemokines play an important role in the regulation of both metastatic properties of malignant cells and initiation of specific antitumor immune responses. However, characterization of biological significance of chemokines expressing by prostate carcinoma has not been fully elucidate. Surprisingly, dendritic cells (DC), which perform an essential role in the generation and regulation of antitumor immune responses, do not efficiently infiltrate the prostate cancer tissue, a step which is crucial for initiation of antitumor immunity. We hypothesized that prostate cancer cells lose expression of a novel dendritic cell attracting chemokine CXCL14, which is normally expressed by virtually all non-malignant tissues, including the prostate gland. The main goal of this proposal is to determine the mechanisms of the regulation of CXCL14 expression by prostate cancer and test whether recovery of CXCL14 expression on tumor cells will be accomplished by attraction of dendritic cells and initiation of effective antitumor immune responses. Since, novel therapies that can correct the dendritic cell system activity without compromising normal host cell-mediated immunity are desirable, identification of mechanisms regulating dendritic cell trafficking and homing in prostate cancer will be critical for the development of the next generation of comprehensive vaccine systems.

Body

Recent research has identified chemokines responsible for neutrophil and monocyte trafficking into inflamed tissues and lymphocyte homing to lymphoid organs. Less was known about the trafficking of DC, particularly the recruitment of DC to the tumor site. A few chemokines, including MIP-3α, MCP-1 and RANTES, have been shown to be expressed in different tumors. However they are not critical determinants of the recruitment of tumor-associated DC. Our data demonstrated that expression of a new DC chemokine CXCL14 was lost in prostate cancer, in association with reduced infiltration of tumors by DC. We speculated that low levels of prostate cancer infiltration by DC may be due to a low or lost expression of CXCL14 in tumor cells, which, in turn, results in low recognition of tumor cells by antigen-presenting DC and in failure to initiate antitumor immune responses. Task 1 for the first year of support focused on *in vitro* studies aiming to characterize CXCL14-induced DC chemotaxis. Thus our OBJECTIVE 1 was to Characterize the chemotactic activity of CXCL14 towards human DC *in vitro.* Specifically, we proposed to determine: (i) expression of CXCL14 chemokine in different human prostate tissues, (ii) chemotactic potential of CXCL14 for human DC, and (iii) attraction of immature versus mature DC towards CSCL14 chemokine.

(i) Expression of CXCL14 chemokine in different human prostate tissues

Immunohistochemical analysis of tumor-infiltrating DC in prostate cancer using CD83 marker for human DC revealed a significant reduction of DC numbers in the tumor tissues when compared to the non-malignant tissues (N=10). BPH specimens, used as an additional control, also demonstrated high levels of infiltrating DC. These data indirectly support our working hypothesis that DC migration into the prostate cancer tissues might be deficient if compared with DC migration to the non-malignant tissues. Next, we tested whether decreased infiltration of PCa by DC may be associated with

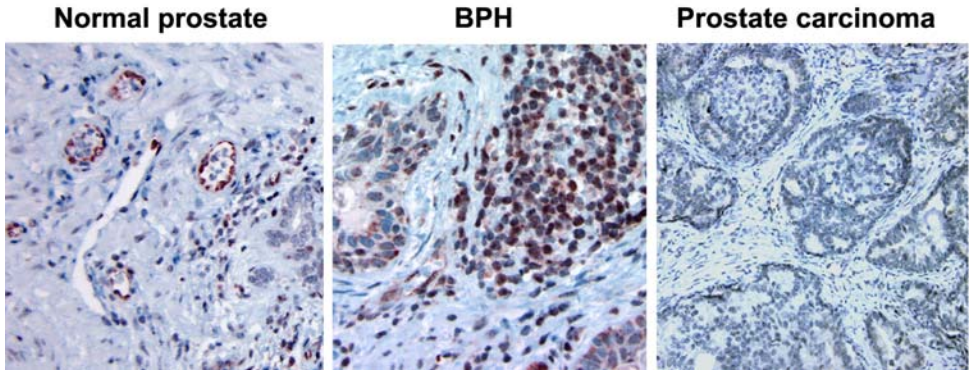

Figure 1. Loss of CXCL14 expression in prostate cancer specimens. Five-μm sections were pretreated with EDTA buffer pH 8.0 in a microwave oven, washed in PBS/Tween20, and stained with anti-CXCL14 antibodies. Biotinylated secondary antibodies were added for 30 min and positive reaction was developed using ABC technique.

decreased expression of certain DC chemokines, we measured expression of CXCL14 protein in different prostate cancer tissues by immunohistochemistry. Our results revealed that normal prostate (N=7) and BPH tissues (N=7) were strongly positive for CXCL14, whereas prostate cancer tissues (N=10) were negative for CXCL14 staining (Fig.1).

Analysis of CXCL14 mRNA expression in prostate cancer cell lines confirmed the down-regulation of CXCL14 expression in malignant cells, whereas normal prostate epithelial cells expressed high levels of CXCL14 mRNA (Fig. 2A). PCa cells obtained from primary human tumor specimens by needle microdissection technique demonstrated lower or no CXCL14 mRNA expression, whereas, adjunct normal prostate cells expressed higher levels of CXCL14 mRNA (Fig. 2B). Figure 2C demonstrates the densitometric analysis of these data shown as pair of prostate adenocarcinoma and adjunct normal prostate tissue. All evaluated specimens showed significantly reduced levels of CXCL14 expression in PCa tissues when compared to the normal adjunct areas. Thus, these data indirectly support the hypothesis that reduced infiltration of PCa by DC may be associated with an absence of expression of DC chemokines such as CXCL14.

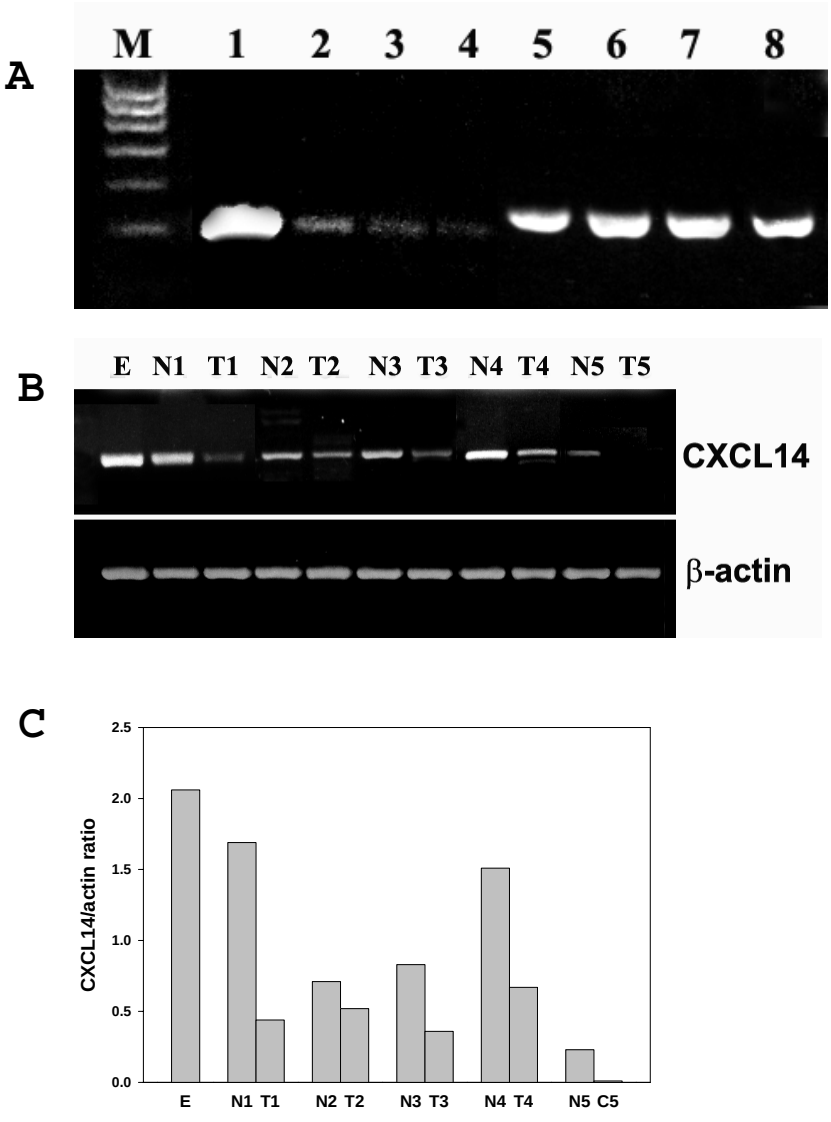

Figure 2. (A). PCa cells display down-regulated expression of CXCL14. Total RNA was extracted from PCa cells. Lanes 2 and 6, LNCaP; Lanes 3 and 7, DU145; Lanes 4 and 8, PC3. Normal prostate epithelial cells served as a positive control (Lanes 1and 5). RT-PCR was carried out to evaluate the expression of CXCL14 (Lanes 1-4) and β-actin (Lanes 5-8) mRNA (313 bp and 323 bp).
(B). Expression of CXCL14 mRNA is down-regulated in prostatic adenocarcinoma in comparison to the benign prostatic glands. Total RNA was extracted from paired malignant glands of adenocarcinoma (T1-T5) and histologically benign prostatic glands (N1-N5). The expression of CXCL14 mRNA was assessed by RT-PCR.
(C). Densitometric analysis of expression of CXCL14 mRNA. Data are presented as pair of prostate adenocarcinoma (T1-T5) and adjunct normal prostate tissue (N1-N5). E, normal prostate epithelial cells served as control. All evaluated specimens showed significantly reduced level of CXCL14 expression in PCa tissue when compared to the normal adjunct areas.

(ii) Chemotactic potential of CXCL14 for human DC

Recently, we demonstrated that CXCL14 and CXCL14-positive HNSCC (Head and Neck Squamouse Cell Carcinoma) cell lines were potent inducers of DC chemoattraction *in vitro*, whereas CXCL14-negative HNSCC cell lines did not chemoattract DC in a Transwell assay. Here, to expand these observations, DC migration was evaluated by a different chemotaxis assay using microwell Boyden chambers: BW200S (Neuroprobe) and polycarbonate filters (5 μm pore size; Osmonics Inc). We determined that CXCL14 is a potent DC chemoattractant *in vitro* (Fig. 3). As shown in Figure 3, CXCL14 induced migration of monocytes-derived DC across polycarbonate filters in a dose-

dependent manner. For example, the number of migrated DC stimulated by 50 ng/ml of CXCL14 was 37.5 ± 5.4, while CXCL14 at concentration of 200 ng/ml increased the DC migration to 63.7 ± 8.9 cells. The spontaneous migration of DC to a control medium was 26.1 ± 8.2 ($p<0.05$, Fig. 3).

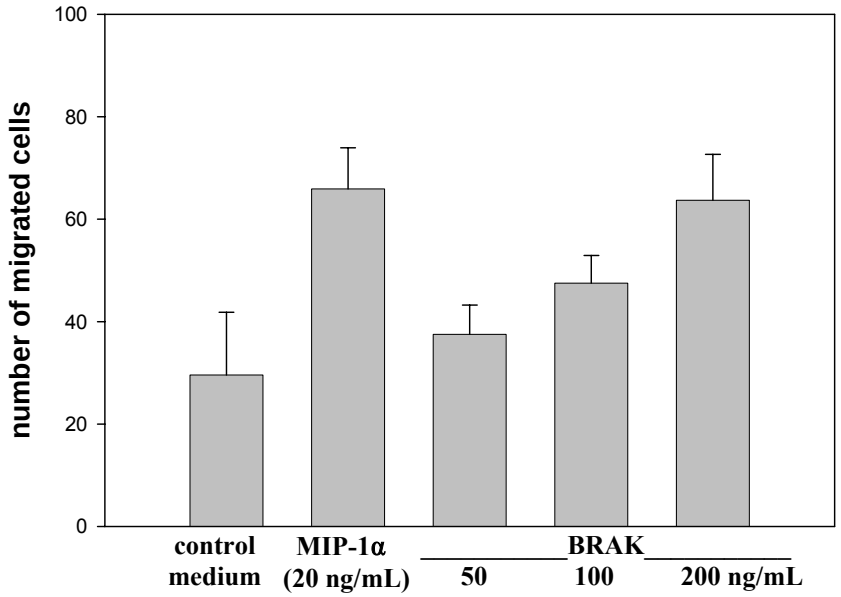

Figure 3. CXCL14 chemokine dose-dependently chemoattracts human DC *in vitro*. DC were generated from monocytes, and their chemotaxis was assessed in Boyden microchambers. MIP-1α served as a positive control. Results are expressed as the mean number of migrated cells in five not-overlapping high-power microscope field from each well. Three experiments have shown similar results. *, $p<0.05$.

(iii) Attraction of immature versus mature DC towards CSCL14 chemokine

Next question was whether CXCL14 chemoattracts both immature and mature DC. CD14-derived DC were generated from PBMC isolated from buffy coats by Ficoll gradient centrifugation. The PBMC were further plated at 10^7 cells/well in 2 ml of AIM V medium (GIBCO) in 6-well plates. After 1-h incubation at 37ºC in a humidified 5% CO_2 atmosphere, non-adherent cells were removed and adherent monocytes were gently washed with warm AIM V medium. Adherent monocytes were cultured with recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF; 1000 U/ml, PeproTech) and IL-4 (1000U/ml, PeproTech) in complete RPMI medium for 7 days. Maturation of DC was stimulated by additional supplementation with 20 ng/ml of tumor necrosis factor-α (TNF-α, PeproTech) on day 6.Figure 4 demonstrates that only immature, but not mature DC, are chemoattracted by CXCL14. These data are in agreement with the general concept that immature DC are attracted to non-lymphoid tissues where a number of potent DC chemokines, including CXCL14, may be ubiquitously expressed.

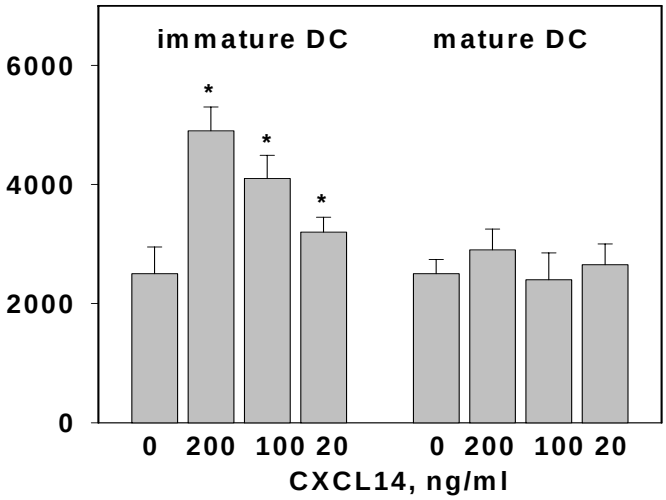

Figure 4. Immature (GM-CSF + IL-4, Day 6), but not mature (GM-CSF + IL-4 + TNF-α, Day 8) DC migrate towards CXCL14. The representative results are shown (Mean ± SEM) (N=4). *, $p<0.05$, (one-way ANOVA and *t*-test).

Key Research Accomplishments

- Prostate carcinoma mass are low infiltrated by dendritic cells, key immunological cells responsible for initiation of antitumor immunity
- Prostate cancer cell lines can be characterized by low expression of CXCL14 chemokine protein determined by Immunohistochemical methods
- Prostate cancer cell lines can be characterized by low expression of CXCL14 chemokine mRNA assessed by RT-PCR. Together with our data showing low expression of CXCL14, these results support the hypothesis that loss expression of certain chemokines within prostate cancer bed may be associated with attraction of immune cells and, thus, deficient antitumor immunity
- Prostate cancer tissue obtained from cancer patients express low levels of CXCL14 chemokine, which confirms the conclusions shown above
- CXCL14 chemokine is a potent chemoattractant for human DC as was shown by two different methods: Transwell insert migration and Boyan microchember migration
- Only immature human DC express receptors for CXCL14 chemokine since mature DC were not chemoattractive to CXCL14 protein in vitro.

Reportable Outcomes

PUBLICATIONS:

1. Shurin M.R., Shurin G.V., Lokshin A., Yurkovetsky Z.R., Gutkin D.W., Chatta G.S., Zhong H., Han B., Ferris R.L. Intratumoral cytokines/chemokines.growth factors and tumor infiltrating dendritic cells: Friends or enemies? *Cancer Metastasis Reviews*, 2006, in press.
2. Song E.Y., Shurin M.R., Tourkova I.L., Chatta G.S., Gutkin D.W., Shurin G.V.Regulation of Dendritic Cell Chemokine CXCL14 Expression in Human Prostate Carcinoma. Submitted.
3. Shurin M.R., Song E.Y., Tourkova I.L., Perez L., Dutkin D.W., Shurin G.V. Epigenetic down-regulation of CXCL14 expression in tumor tissues is associated with low attraction of dendritic cells both in vitro and in vivo. Keystone Symposia on Chemokines and Chemokine Receptors, p.79. Snowbird, Utah, 2006.

PRESENTATIONS:

- Shurin M.R. “Epigenetic mechanisms of immune escape”. Department of Pathology Seminar Series. Pittsburgh, PA. December 2005.
- Shurin M.R. “Tumor cells and dendritic cells: How to break the survival of the fittest”, Immunology lecture series, Pittsburgh, PA. April 2006.

Conclusions

During the first year of support, we developed a marked progress toward the main goal of our proposal – understanding the mechanisms of chemokine regulation in prostate cancer. Specifically, we revealed that prostate cancer cell lines and tissues obtained from cancer patients express low or no CXCL14 chemokine protein and mRNA, which might results in low infiltration of the tumor mass by dendritic cells. Importantly, if dendritic cells are not attracted to the prostate cancer tissues, no antitumor immune responses may be generated due to the absence of tumor antigen recognition, processing and presentation. These fundamental findings will now allow us to move forward and investigate the biological significance of these findings and the mechanisms of CXCL14 regulation in tumor cells.

References

Appendices