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Serum alleviates the lung cancer induced inflammatory responses from monocytes

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Abstract: **Tumor-associated macrophages constitute the preponderant inflammatory immune infiltrate in solid tumors and the cytokines released from them possess diversified significance in tumor development. Lung cancer cells have been shown to stimulate the macrophages to release various cytokines like TNF-α and IL-6. TNF-α, in particular, has been shown to cause metastasis in mice, however, the process has not been extensively analyzed in human lung cancers. In order to investigate these responses in human lung cancers, we used human adenocarcinoma cell line A549 and human monocytic cell line THP-1 to study the process in detail. Stimulation of THP-1 cells with the conditioned medium of A549 cells causes the release of TNF-α and the co-culture of the two cell types results in the increased migratory abilities of A549 cells *in vitro*. Stimulation carried out in the presence and absence of serum established that serum could alleviate the stimulatory effects of A549 cells.**

Keywords: **Lung cancer, TNF-α, Serum, Metastasis.**

I. INTRODUCTION

The tumor microenvironment is dynamic being in a state of constant evolution. It undergoes tissue remodelling, metabolic changes and recruits cells of various types notably the immune cells. Among the infiltrate, the myeloid cells constitute the major population in the tumour microenvironment [1]. Myeloid cells are the first line of defence against pathogens but act as a double-edged sword by aiding the tumor growth [2]. It augments the metastatic ability of the tumors by preparing pre-metastatic niches [3] [4]. This pathological camaraderie between myeloid and cancer cells is complex but use common mediators/pathways to regulate immune response and go hand-in-hand with angiogenesis [5]. There is a constant and active interaction between the two cell populations; however, the precise nature of this dynamic interaction is far from clear. Therefore, we investigated the nature and modulation of this interaction. Cell enriched supernatants from *in vitro* cultures of lung cancer cells are known to stimulate macrophage cells to release TNF-α through TLR-2 stimulation that leads to recruitment of myeloid cells and possibly promoting metastasis [6] [7]. With growing evidences regarding the role of inflammation-induced cell migration and invasion, anti-inflammatory approaches are being tested for cancer prevention and therapy [8] [9] [10]. We first confirmed the stimulation of monocytic cell line THP-1 with the adenocarcinoma cell line A549 to release the inflammatory cytokine. The cells as well as the conditioned media from the adenocarcinoma cell line triggered the release of TNF-α. We show that serum can alleviate this response by possibly preventing the release of TNF-α. However, the inhibitory effect of serum was not observed when THP-1 cells were stimulated with Pam3csk, an artificial TLR-2 ligand indicating that the inhibitory effect of serum may be particular for A549 stimulus rather than being a general response.

II. METHODOLOGY

A. Cell culture

A549 human lung adenocarcinoma cells were cultured in a 37°C, 5% CO2 humidified incubator in RPMI medium, supplemented with 10% Fetal Bovine Serum. A549 cells were passaged at 70-80% confluence using 1X Trypsin-EDTA. THP-1 cells were cultured in RPMI medium supplemented with 10% Fetal Bovine Serum.

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B. Enzyme Linked Immunosorbent Assay

Microtitre plate was coated with TNF-α capture antibody (50µl/well) and left overnight at 4°C. Next day, the plate was washed three times with PBS-Tween and blocked with 1% PBS-BSA for 1hr. The plate was washed after 1hr and 50µl cell soup was added in each capture antibody coated well and incubated for 1hr at 37 °C. The plate was washed three times with PBS-Tween and incubated with detection antibody for 1hr. After 1 hour, plate was washed and incubated with substrate until the development of blue colour. Reading was taken at 450nm.

C. Wound healing Assay

A549 cells were cultured in RPMI medium and grown to confluence. Cells were scratched with a P1000 tip to create a wound. Cells were co-cultured with THP-1 cells. TNF-α was used as a positive control. Cells were timely monitored under microscope for wound healing.

III. RESULTS AND DISCUSSION

Lung cancer cell lines such as mouse Lewis Lung Carcinoma (LLC) have been shown to stimulate macrophages to release TNF-α which causes metastasis in lung cancer cells. Human lung adenocarcinoma cell line A549 also elicits similar effects. We cultured A549 cells in RPMI-10 medium and collected the conditioned medium. A549 cells and the conditioned medium were used to stimulate human monocytic cell line THP-1 along with artificial TLR-2 ligand, Pam3csk, as a positive control for 6 hours and TNF-α levels were measured by ELISA. As expected, the stimulation of the cells lead to the release of TNF-α. In order to analyse the effect of TNF-α release on the migration of A549 cells, we performed the wound healing assay. Cells were grown to confluence and scratched with a P100 tip to create a wound. Cells were co cultured with THP-1 cells to observe their effect on the mobility of A549 cells to the wound, which signifies cell migration. TNF-α was used as a control. Cells were timely monitored under microscope for wound healing. We observed that co-culture of lung cancer cells and monocytic cells increased the migratory ability of the A549 cells (Figure 1). TNF-α alone was also sufficient to induce the migration of the cells implying that it might be the migration-promoting factor.

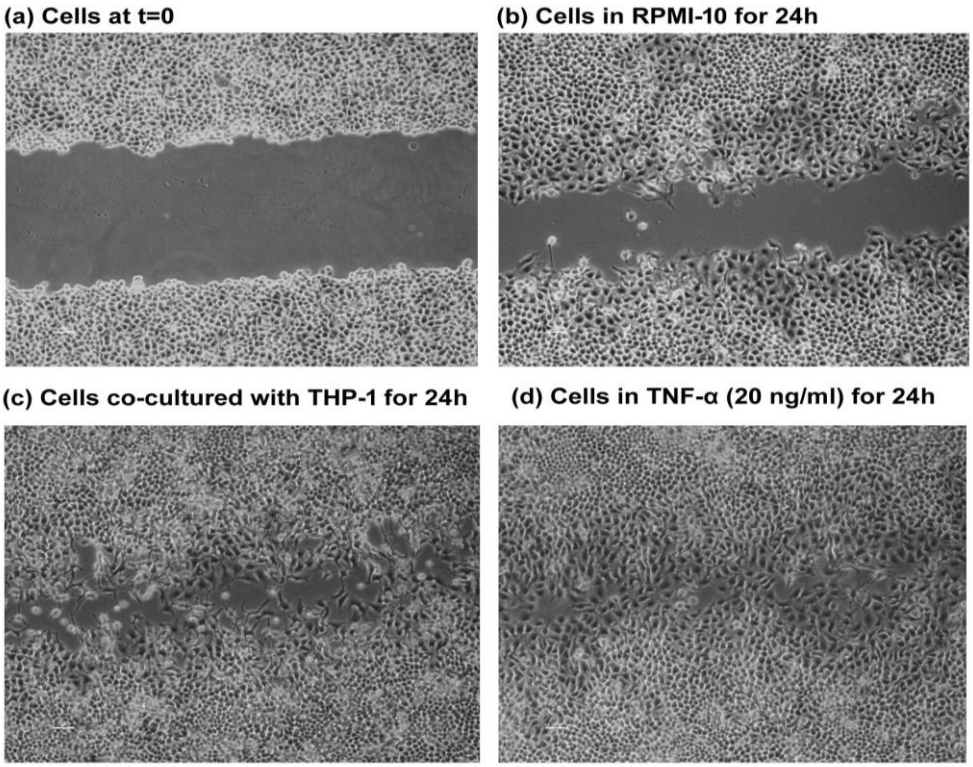

Figure 1. Release of TNF- α from THP-1 cells may cause migration of A549 cells *in vitro*. Cells were grown to confluence and scratched with a tip. Cells were co cultured with THP-1 cells or treated with TNF-α. Images were taken in a Nikon microscope attached with a digital camera. The experiment was repeated two times with very similar results

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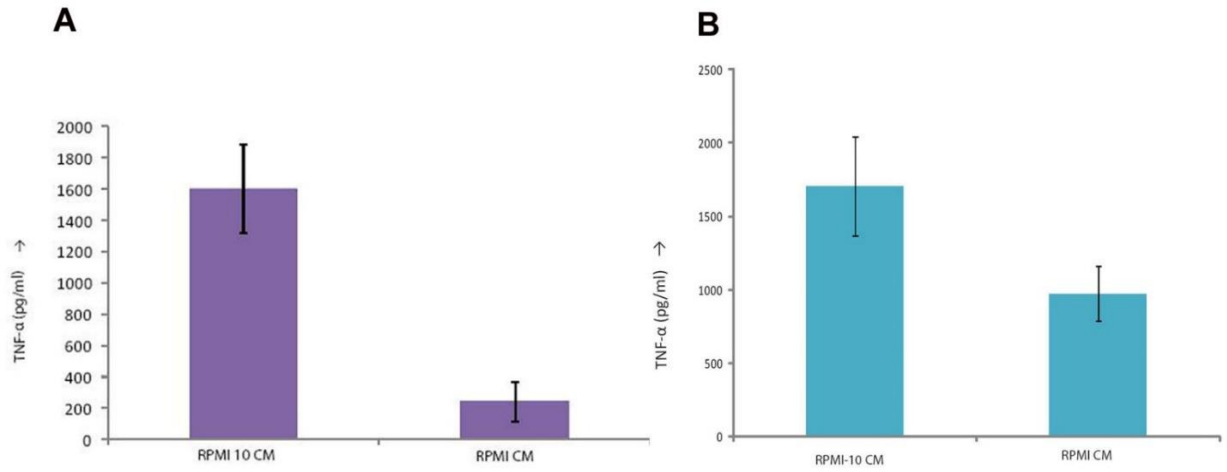

Figure 2. Serum inhibits the stimulation of THP-1 cells with A549 conditioned media: Conditioned medium was collected from A549 cells grown in the presence of serum and the cells not supplemented with any serum. These conditioned media were used to stimulate THP-1 cells. The THP-1 cells were re-suspended in (a) presence and (b) absence of serum. TNF-α levels were measured after 6h. The experiment was repeated two times with very similar results; CM-conditioned media.

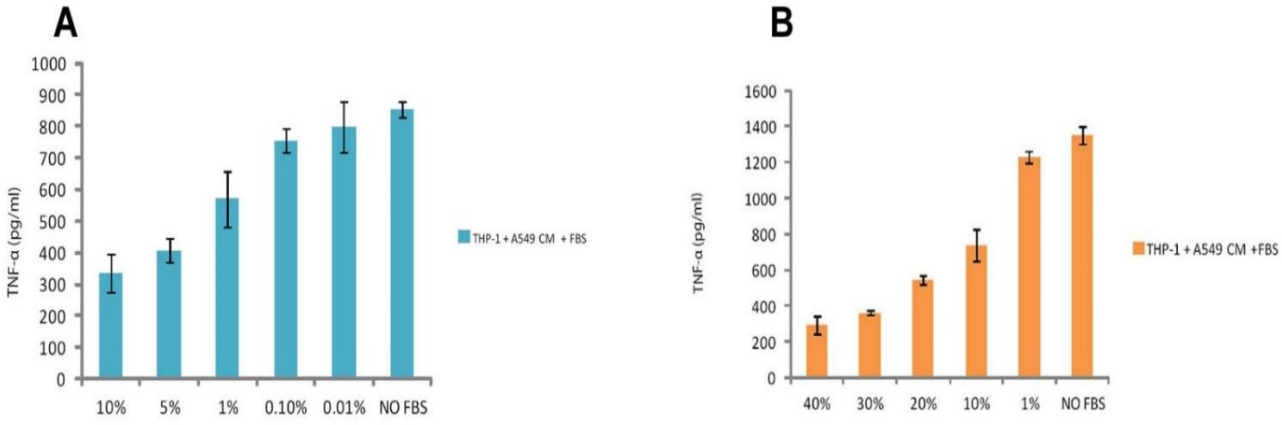

Figure 3. Serum inhibits the responses from both serum containing and serum free conditioned media: THP-1 cells were stimulated with conditioned media collected from A549 cells grown in (a) RPMI and (b) RPMI-10 media, in the medium supplemented with various concentrations of FBS. The experiment was repeated two times with very similar results; CM- Conditioned media, FBS-fetal bovine serum.

Serum plays a critical role in producing the stimulating factor from A549 cells. This is evident from the very poor response of conditioned medium taken from A549 cells grown under serum-free conditions as compared to conditioned medium collected from cells grown in serum (10% fetal bovine serum) supplemented medium. We wondered what role serum might play during the stimulation of THP-1 cells. We collected the conditioned media from A549 cells grown in serum supplemented as well as serum-starved conditions. THP-1 cells were then stimulated with these media (Figure 2). The stimulations were also carried out in different concentrations of the serum. Serum was able to counter the stimulation of THP-1 by conditioned medium more effectively with increased concentrations. The inhibitory effect of serum was not observed when THP-1 cells were stimulated with Pam3csk, an artificial TLR-2 ligand implying that this response is not general but specific to lung cancer stimulus.

IV. CONCLUSION

Stimulation of THP-1 cells either with A549 cells or its conditioned medium results in the release of the pro-inflammatory cytokine TNF-α. Contact co-culture between THP-1 and A549 cells induced the metastatic behaviour in A549 cells. Serum was able to counter the stimulation of THP-1 cells, which may prevent the process of metastasis.

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