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STUDY ON INTERACTION OF IMATINIB IN LUNG CANCER WITH TUMOR ASSOCIATED MACROPHAGES

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Abstract:

Introduction: *Imatinib is a tyrosine kinase inhibitor and firstly used for Philadelphia chromosome-positive (Ph^+) chronic myelogenous leukemia (CML). It has also been found that Imatinib has therapeutic effect on several solid tumors.* ***Aims and objectives:*** *The basic aim of the study is to find the interaction of Imatinib in lung cancer with tumor associated macrophages.* ***Methodology of the study:*** *This study was conducted at DHQ hospital Khanewal during June 2018 to August 2018 with the permission of ethical committee of hospital. Imatinib mesylate was obtained from Sigma-Aldrich and its solutions were prepared at the stock concentration of 50 mM (DMSO). Recombinant murine IL-13, IL-4 and M-CSF were purchased from PeproTech. LPS was also purchased from Sigma-Aldrich. Total RNA from RAW264.7 cells and BMDMs was isolated using Trizol (Invitrogen, CA, USA), and cDNA was synthesized using TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix.* ***Results:*** *Given that STAT6 plays a key role in IL-13 or IL-4 induced macrophage M2-like polarization, we assessed whether STAT6 is involved in Imatinib inhibited M2 polarization. IL-13 markedly triggered the phosphorylation of STAT6 in 30 min, and reached a peak in 1 h, which was significantly restrained by Imatinib.* ***Conclusions:*** *It is concluded that Imatinib inhibits macrophage M2-like polarization both in vitro and in vivo which contributes to its anti-metastatic function in lung cancer.*

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INTRODUCTION:

Imatinib is a tyrosine kinase inhibitor and firstly used for Philadelphia chromosome-positive (Ph^+) chronic myelogenous leukemia (CML). It has also been found that Imatinib has therapeutic effect on several solid tumors. For example, the anti-metastasis function of Imatinib is reported in breast cancer, lung cancer and dermato fibrosarcoma protuberans when it is used alone or combined with other anti-tumor agents. However, controversial results come out that Imatinib increases lung metastasis in breast tumor grafted murine models. The published studies which focused on inhibition of cell proliferation or induction of cell apoptosis, are not able to fully explain of Imatinib in cancer therapy [1].

Macrophages are essential components of the inflammatory microenvironment of tumors. Conventional treatment modalities (chemotherapy and radiotherapy), targeted drugs, antiangiogenic agents, and immunotherapy, including checkpoint blockade, all profoundly influence or depend on the function of tumor-associated macrophages (TAMs). Chemotherapy and radiotherapy can have dual influences on TAMs in that a misdirected macrophage-orchestrated tissue repair response can result in chemo resistance, but in other circumstances, TAMs are essential for effective therapy [2]. A better understanding of the interaction of anticancer therapies with innate immunity, and TAMs in particular, may pave the way to better patient selection and innovative combinations of conventional approaches with immunotherapy [3].

Accumulating evidence suggests that whether macrophages execute tumor-promoting or tumor-suppressing activities depends on their sub-phenotype which is dynamically switched. Macrophage activation is broadly categorized as classically activated, or M1, and alternatively activated, or M2 in response to different micro environmental stimuli. M1-like polarization of macrophages are activated by toll-like receptor agonists (e.g., LPS) and Th1 cytokines (e.g., INFγ) with enhancing abilities of phagocytosis, pro-inflammatory cytokines production (e.g., TNFα), and tumor destruction [4]. Alternatively, M2-like polarization of macrophages, which produce secretory factors to promote angiogenesis, matrix remodeling, and tumor progression, is induced by Th2 cytokines, like IL-13 and IL-4 [5]. This M1/M2 concept helps to explain macrophage heterogeneity. To more accurately characterize the complexity of M2-like macrophage activation. TAMs in malignant tumors tend to resemble alternatively activated macrophages (M2-like), which enhance tumor-associated angiogenesis, promote the ability of tumor migration and invasion, as well as suppress the anti-tumor immune responses [6].

Aims and objectives

The basic aim of the study is to find the interaction of Imatinib in lung cancer with tumor associated macrophages.

METHODOLOGY OF THE STUDY:

This study was conducted at DHQ hospital Khanewal during June 2018 to August 2018 with the permission of ethical committee of hospital. Imatinib mesylate was obtained from Sigma-Aldrich and its solutions were prepared at the stock concentration of 50 mM (DMSO). Recombinant murine IL-13, IL-4 and M-CSF were purchased from PeproTech. LPS was also purchased from Sigma-Aldrich. Total RNA from RAW264.7 cells and BMDMs was isolated using Trizol, and cDNA was synthesized using TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix. The real-time PCR assay was performed by SYBR Green Master Mix. The reaction mixtures were prepared following manufacture's protocol.

Table 1: Sequences of Primers.

Primer	Sequence (5′-3′)	
Mrc1	Forward	AAGGCTATCCTGGTGGAAGAA
	Reverse	AGGGAAGGGTCAGTCTGTGTT
Arg1	Forward	CCACAGTCTGGCAGTTGGAAG
	Reverse	GGTTGTCAGGGGAGTGTTGATG
CDH1	Forward	CAGCCTTCTTTTCGGAAGACT
	Reverse	GGTAGACAGCTCCCTATGACTG
MMP9	Forward	TTCGACTTGAAGTCTCAGAAGGTG
	Reverse	ATGGCAGAAATAGGCTTTGTCTTG
CCL2	Forward	GATGCAGTTAACGCCCCACT
	Reverse	ACCCATTCCTTCTTGGGGTC
iNOS	Forward	TTTGCTTCCATGCTAATGCGAAAG
	Reverse	GCTCTGTTGAGGTCTAAAGGCTCCG

Statistical analyses

All samples were analyzed by at least three independent experiments. Data were analyzed by Student's t-test and are expressed as mean ± SD. Differences were statistically significant when the p value was less than 0.05.

RESULTS:

Given that STAT6 plays a key role in IL-13 or IL-4 induced macrophage M2-like polarization, we assessed whether STAT6 is involved in Imatinib inhibited M2 polarization. IL-13 markedly triggered the phosphorylation of STAT6 in 30 min, and reached a peak in 1 h, which was significantly restrained by Imatinib (Figure 01). Furthermore, the translocation of STAT6 into nuclear promoted by IL-13 was suppressed by Imatinib. Because STAT6 is classical activated by JAK2, we analyzed JAK2 phosphorylation upon IL-13 or IL-4 stimulation and/or Imatinib treatment. We found that IL-13 or IL-4 stimulated JAK2 phosphorylation was not affected by Imatinib, which suggested that Imatinib inhibited the phosphorylation of STAT6 through JAK2-independent pathway.

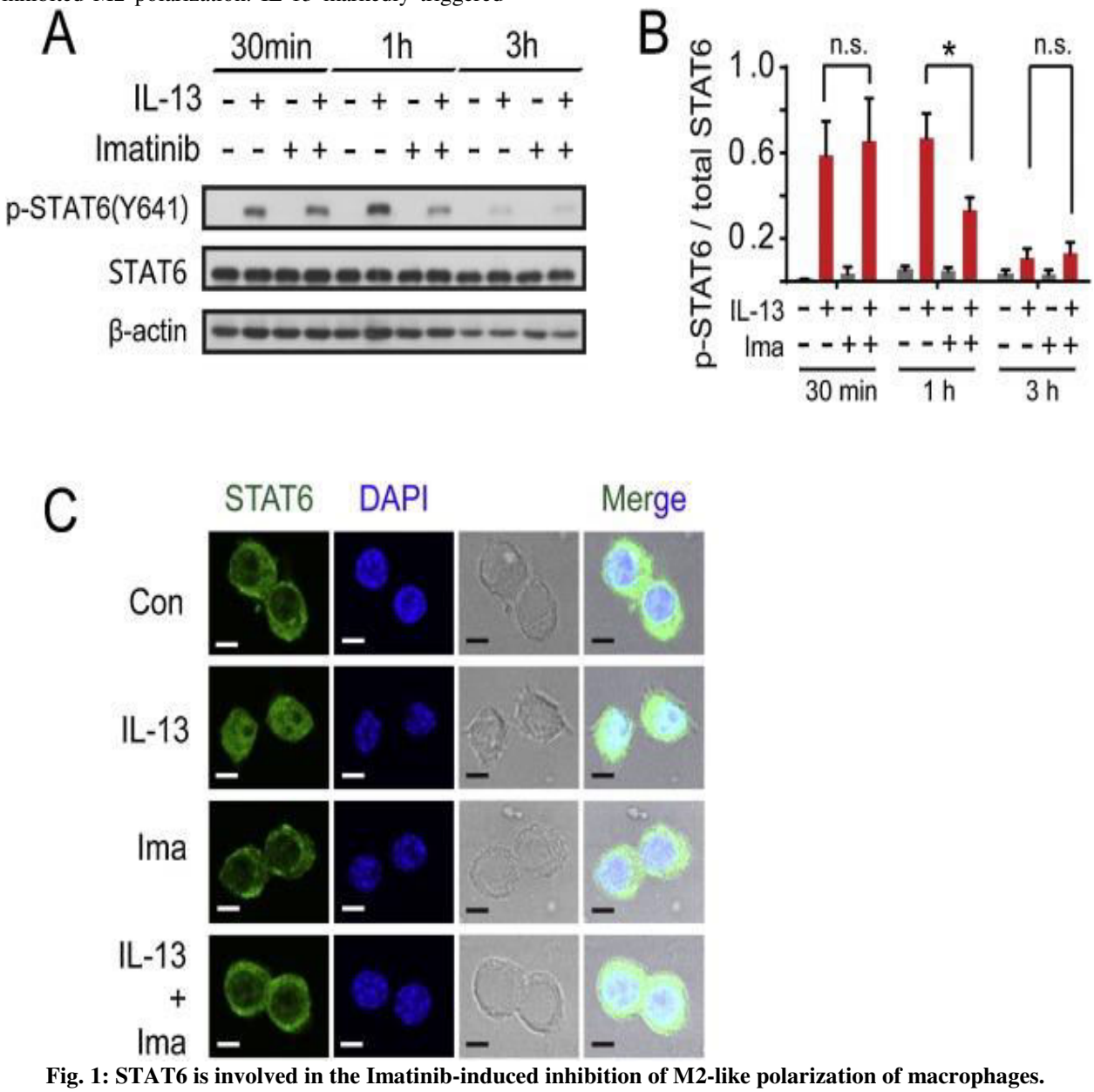

Fig. 1: STAT6 is involved in the Imatinib-induced inhibition of M2-like polarization of macrophages.

DISCUSSION:

Imatinib mesylate, the first tyrosine kinase inhibitor approved in clinic use, has showed remarkable activity against Ph^+ hematological malignancies and metastatic solid tumors, especially GIST. Imatinib is widely used in KIT^+ GIST, due to its inhibition of c-kit, a kind of tyrosine kinase enzymes[7]. It has been illustrated that Imatinib inhibited bone metastasis of breast cancer by the blockade of CSF1R signals. Several studies showed that Imatinib has a therapeutic effect on colorectal metastasis and proliferation of tumor cells via abrogating PDGFR signaling pathway. Whereas, opposing effect of Imatinib as a PDGFR inhibitor is also reported. It may attribute to its impact on PDGF-β signaling in pericytes from PDGF-BB-low-producing or PDGF-BB-negative tumors. The mechanism of anti-metastasis of Imatinib remains unclear. Furthermore, there are also studies showed that the anti-tumor activity of Imatinib partially depended on immune cells like T cells[8]. As TAMs are the most abundant immune cells in tumor microenvironment which contribute to tumor malignancy, we investigated the effect of Imatinib on polarization of TAMs and tumor metastasis.

M2-like polarization of macrophages is driven by IL-4 and IL-13 secreted by immune cells like T_H2 cells[9]. By the employment of IL-13 or IL-4 induced M2 polarization models, we found that Imatinib efficiently suppressed the expression of prototypical M2 marker CD206 and M2 genes. Furthermore, we also confirmed that the conditioned medium of M2-like macrophages induced by IL-13 promoted the migration of LLC cells, as the previous study reported. Meanwhile, the migration of LLC cells was attenuated when incubated with the conditioned medium of BMDMs treated with Imatinib and IL-13 [10]. However, there was no significant difference between the migration of LLC cells treated directly with IL-13 and/or Imatinib. Therefore, Imatinib can diminish tumors migration through the inhibition of M2 macrophage polarization induced by IL-13 or IL-4 [11].

Blocking macrophage recruitment and survival has been extensively investigated in preclinical models and is undergoing clinical evaluation. TAMs typically originate from blood monocytes that are continuously recruited from the circulation, although a certain degree of self-renewal in some tumors has been reported. Among chemoattractants that regulate the influx of circulating monocytes in tumor tissue, chemokines have been extensively studied, in particular CCL2. Antibodies to CCL2 are now being tested in clinical trials. Combinations of carlumab with conventional chemotherapy regimens are being studied in clinical trials. Recent results caution against the possibility that interruption of anti-CCL2 therapy may lead to enhanced metastasis. In a breast cancer model, cessation of anti-CCL2 therapy was associated with monocyte release from the bone marrow, increased mobilization and infiltration of cancer cells, and angiogenesis driven by IL-6 and VEGF. In addition, recent results suggest that complement components are key players in cancer-related inflammation and orchestrate macrophage recruitment in part via CCL2. Thus, targeting complement with available tools (e.g., anti-C5a) should be taken into consideration [12].

CONCLUSIONS:

It is concluded that Imatinib inhibits macrophage M2-like polarization both in vitro and in vivo which contributes to its anti-metastatic function in lung cancer. Furthermore, we identify STAT6 plays a vital role in the effect of Imatinib on M2-like polarization of macrophages.

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