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TLR7 and TLR8 agonist resiquimod (R848) differently regulates MIF expression in cells and organs

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ABSTRACT

Since its first description in 1966, macrophage migration inhibitory factor (MIF) was found to play a critical role in inflammatory and immune responses as well as in disease pathogenesis especially in tumor pathogenesis and cancer progression. MIF is expressed in different cell types and is associated with many disease severity and tumor pathogenesis. Here, we investigated the influence of TLR7 and TLR8 agonist resiquimod (R848), an immune response inducer used as a prophylactic agent for several infectious diseases as well as anticancer agents and vaccine adjuvant on MIF expression in cells and organs. Humans, mice and rats cell lines from different tissues (blood, retinal, nasopharynx, brain and liver) and C57BL/6J mice organs (brain, liver and spleen) were used for this investigation. *In vitro*, R848 induced MIF gene overexpression except in brain and liver cells. Furthermore, it enhanced cells ability to release soluble MIF and differently regulated mRNA expression of MIF-related receptors (*CD74, CXCR4, CXCR2 and CD44*). Its influence on *MIF* gene expression and MIF proteins release was more consistent in cancer cells. *In vivo*, a strong positive expression of MIF was observed in different regions in brain and spleen in response to R848 treatment; however in liver, increased MIF expression was observed in hepatocytes only. On the other hand, R848 treatment had induced a slight enhancement of MIF concentration in the plasma of C57BL/6J mice. Taken together, these data suggest that R848 differently regulates *MIF* mRNA expression depending on organ types and could influence MIF concentration in cellular microenvironment.

1. Introduction

Since its first description in 1966 [1,2], MIF was found to play a pivotal role in inflammatory and immune responses, in disease pathogenesis especially in tumor pathogenesis and cancer progression as well as in neuroendocrine axis [3–6]. It is highly expressed in many types of cancer and its implication in tumorigenesis and aggressiveness is mainly due to its interaction with receptors CD74, CXCR4, CXCR2 or CD44 [5–7]. Intracellularly, MIF interacts with a wide board of partner proteins to regulate key pathways involved in essential cellar systems such as p53-mediated senescence and apoptosis, redox balance [8,9], Jab1/CSN5-mediated genes expression and cell cycle control [10] as well as in multitude of signaling pathways.

Unlike most cytokines, MIF is produced in various organ by different cells including T cells, monocytes, macrophages, blood dendritic cells, B cells, neutrophils, eosinophils, mast cells, basophils and epithelial cells [11,12]. It can be released in response to various stimuli including stress, depressive symptoms [13], viral proteins [14] or by toll like receptor (TLR) stimulation [15].

Cancer and infectious diseases are among the leading causes of death all over the world. On this purpose, researches are carried out to find the right treatment to fight against these pathologies. Nowadays, R848 is among drugs test in cancer therapy [16–19] and it was also described for its antiviral properties [16,20,21]. It belongs to imidazoquinolinamines compounds and has an immunomodulatory effect. Signaling through TLR7 and TLR8 pathways, R848 leads to the production of pro-inflammatory cytokines such as TNF, IFN-α, IL-1β, IL-6, IL-8, chemokines, type I interferons and the up-regulation of co-stimulatory molecules [22,23]. However, its influence on the regulation of MIF expression remains misunderstood. In this present study, we investigated in cell lines and organs, the influence of R848 on MIF mRNA and proteins expression and R848 impact on its related-receptors

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mRNA expression.

2. Materials and methods

2.1. Mice

Six to eight weeks C57BL/6J mice were purchased from Charles River Laboratories (China). This study was carried out in strict accordance with the National Institutes of Health guidelines for the care and use of laboratory animals as recommended by Chinese regulations for the administration of affairs concerning experimental animals. The protocol used was approved by the experimentation ethics committee on animal rights protection of Peking Union Medical College Hospital. 30% choral hydrate (Tianjin Kermel Chemical Reagent Co., Ltd., Tianjin, China) was used for anesthesia.

2.2. Cell lines

Nine cell lines from different tissues including B-cells (n = 3), epithelial cells (n = 5) and fibroblast (n = 1) were used to evaluate the influence of R848 on MIF and its receptors genes and proteins expression. B-cell lines were composed of Ramos, IM9 cell lines (both from China infrastructure of cell line resource) and B958 cell line donated by Viral and Pharmaceutical Research Institute of Beijing University of Technology. The epithelial cells included human normal retinal pigment epithelial cell line D407, human nasopharyngeal carcinoma (NPC) cell line CNE-2Z, human glioblastoma cell line U87 (all purchased from China infrastructure of cell line resource), human hepatocellular carcinoma epithelial cell line HepG2 (purchased from Beijing Cotimes Biotech Co., Ltd) and rat liver epithelial cell line IAR-20 (from Peking union medical college cell resource center). The used fibroblast was a rat glial cell line C6, provided by China infrastructure of cell line resource.

2.3. Cell culture

B-cells, CNE-2Z and D407 cells were cultured in RPMI-1640 (1x) containing L-Glutamine, (gibco® by life technology™); HepG2, IAR-20, U-87 and C-6 cells cultured in DMEM/High glucose (1×) containing 4 mM L-Glutamine and 4500 mg/L of glucose (HyClone®, Thermo Scientific). RPMI-1640 (1×) and DMEM/High glucose (1×) were supplemented with 10% of Fetal Bovine Serum (gibco® by life technology™) and 1% of antibiotic composed of 10,000 Units/ml Penicillin and 10,000 µg/ml Streptomycin (gibco® by life technology™).

2.4. Stimulation with R848

Cells were stimulated with 6 µg/ml R848 in 5% CO_2 at 37 °C. For *in vivo* assay, two groups of mice were established: the first group (control group) had received an intraperitoneal injection of 100 µl of buffer (PBS 1X) used to prepare the R848 injection dose and the second group (Test group) an intraperitoneal injection of 100 µl of the same buffer containing 100 µg of R848 as previously described [24–26]. The mice were sacrificed 24 h later and plasma and organs were collected.

2.5. RT-qPCR

Cells and mice tissues mRNA were extracted using RNeasy Mini Kit (QIAGEN) and cDNA synthetized following ProtoScript® First Strand cDNA Synthesis Kit protocol (New England Biolabs Inc.). PCR reaction mix was prepared using Maxima SYBR Green/ROX qPCR Master Mix (2X) (Thermo Scientific) and real time PCR carried out using an equal amount of cDNA. Thermal cycling condition were consisted of one cycle template denaturation at 95 °C for 10 min followed by 40 cyclic amplifications at 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s. Primers used for detections were listed in the Table 1 below. GAPDH was used for normalization. Relative gene expression and fold change (related to control group: untreated cells or mice) were calculated on the basis of two or three independent replicates using online QIAGEN GeneGlobe

Table 1
List of primers used for MIF and its related receptors mRNA amplification by RT-PCR.

Human target genes	Forward (5′ to 3′)	Revers (5′ to 3′)	References
MIF[a]	CTCTCCGAGCTCACCCAGCAG	CGCGTTCATGTCGTAATAGTT	[27]
CD74[a]	TGACCAGCGCGACCTTATCT	GAGCAGGTGCATCACATGGT	[28]
CXCR4[a]	AGGGGATCAGTATATACACTT	TGCCCACAATGCCAGTTAAG	[29]
CXCR2[a]	AGGCACAGTGAAGACATCGG	CAGCAGGCTCAGCAGGAATA	[30]
CD44[a]	GTGATGGCACCCGCTATG	ACTGTCTTCGTCTGGGATGG	[31]
TLR7[a]	AAACTCCTTGGGGCTAGATG	AGGGTGAGGTTCGTGGTGTT	[32]
TLR8[a]	CAGAGCATCAACCAAAGCAA	GCTGCCGTAGCCTCAAATAC	[33]
GAPDH[a]	ACACCCACTCCTCCACCTTT	TAGCCAAATTCGTTGTCATACC	[34]
Mouse target genes			
MIF	CCATGCCTATGTTCATCGTG	GAACAGCGGTGCAGGTAAGTG	[35]
CD74	ATGACCCAGGACCATGTGAT	CCAGGGAGTTCTTGCTCATC	[35]
CXCR4	GTGCAGCAGGTAGCAGTGA	GTAGATGGTGGGCAGGAAGA	[35]
CXCR2	TCACAAACAGCGTCGTAGAAC	TATGCACACAAACTTGACCAA	[35]
CD44	TTACCCACCATTGACCAAAT	GGTCACTCCACTGTCCTGGT	[35]
TLR7	AATCCACAGGCTCACCCATA	CAGGTACCAAGGGATGTCCT	[36]
TLR8	GACATGGCCCCTAATTTCCT	GACCCAGAAGTCCTCATGGA	[36]
GAPDH	TGAACGGGAAGCTCACTGG	TCCACCACCCTGTTGCTGTA	[35]
Rat target genes			
MIF	TGCCAGAGGGGTTTCTCTC	CGCTCGTGCCACTAAAAGTC	[37]
CD74	AGCGCGACCTCATCTCTAAC	TACAGGACTCCACGGTTGC	[37]
CXCR4	CGTCGTGCACAAGTGGATCT	GTTCAGGCAACAGTGGAAGAAG	[38]
CXCR2	GCAAACCCTTCTACCGTAG	AGAAGTCCATGGCGAAATT	[39]
CD44	GCGAGGCTTTCAACACAACC	ACTCCACTGTCTTGATTCCC	[40]
TLR7	CTGTGTGGTTTGTCTGGTGG	CACTTTGACCTTTGTGTGCG	[41]
TLR8	CCGTCACTGACTGGGTGATCA	CCTCTAAGCAAAGGAGGACACTTTT	[41]
GAPDH	GTTACCAGGGCTGCCTTCTC	GGGTTTCCCGTTGATGACC	[42]

[a] These primers were also used to analyze gene expression in the Tamarin derived B958 cells due to their high similarity with their human homologues genes (refer to supplementary file 2).