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THE ROLE OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION FACTOR 1 IN THE ARTERIOGENESIS PROCESS

Atherosclerosis, the morphological correlates of vascular disease, is characterized by early endonthelial dysfunction, vascular inflammation together with build-up of lipids, cholesterol, calcium and cellular debris within the intima of the vessel wall. This build-up leads to the formation of advanced atherosclerotic plaque *(Hans A.R.Bluyssen,et al., 2012)*. Despite the fact that better treatments have relieved the number of deaths from atherosclerosis-related diseases, and have improved the quality of life for people who have these diseases, atherosclerosis remains the underlying cause of about 50% of all death in westernized society[1], [2]. According to the data given by WHO (the World Health Organization), an estimated 17.9 million people died from Cardiovascular Diseases (CVDs) in 2016, representing 31% of all global deaths. Of these deaths, 85% are due to heart attack and stroke. Over three quarters of CVD deaths take place in low- and middle-income countries, including Uzbekistan. Out of the 17 million premature deaths (under the age of 70) due to noncommunicable diseases in 2015, 82% are in low- and middle-income countries, and 37% are caused by CVDs. Despite the many novel insights from a hematologic, genetic, and pharmacological research, currently available treatments are still not very effective and atherosclerosis remains a common health problem and is still a major burden on humanity. This means that the search for new therapeutical agents is necessary.

The pro-inflammatory cytokine interferon (IFN)-γ, derived from T-cells, is vital for both innate and adaptive immunity and is also expressed at high levels in atherosclerotic lesions. Evidence that IFNγ is necessary and sufficient to cause vascular remodeling is supported by mouse models of atheroma formation, as the serological neutralization or genetic absence of IFNγ markedly reduces the extent of atherosclerosis [3], [4], [5], [6]. The signal transduction pathway initiated by binding of IFNγ to its receptor leads to intracellular phosphorylation of signal transducer and activator of transcription (STAT)1. Subsequently, STAT1 homodimerizes and translocates into the nucleus where it binds to IFNγ-activated sequences (GAS elements) in the promoters of IFNγ-inducible genes or at other sites by further interaction with other transcription factors [7], including mem-

bers of the Interferon Regulatory Factor (IRF) family [8], [9]. Thus, STAT1 plays a major role in mediating immune and pro-inflammatory responses. As such, IFNγ is considered to participate in promoting atherogenic responses through STAT1-mediated "damaging" signals, regulating the functions and properties of all cell types present in the vessel wall. Indeed, Agrawal et al. revealed that STAT1 positively influences lesion formation in experimental atherosclerosis in vivo and is required for optimal progression of foam cell formation in macrophages in vitro and in vivo [10]. However, the specific role for STAT1 in human atherosclerosis has not been previously reported.

This study provides evidence that in HMEC STAT1 coordinates a platform for cross-talk between IFNγ and TLR4, and identifies a STAT1-dependent gene signature that reflects a pro-atherogenic state in coronary artery disease (CAD) and carotid atherosclerosis.

Real-time Polymerase Chain Reaction (PCR) was used in order to analyze and compare the expression of selected target genes. The expression levels were compared with present RNA-sequencing data per each gene and each treatment condition. Human Microvascular Endothelial Cells (HMEC) were provided by the Center for Disease Control and Prevention (Atlanta, GA) and cultured in MCDB-131 medium(IITD PAN, Wroclaw, Poland) containing 10% of fetal bovine serum (FBS) (Gibco, Thermo Fisher), 100 U/ml penicillin, 100 μg/ml streptomycin, 0.01 μg/ml EGF, 0.05 μM hydrocortisone and 2 mM L-glutamine. At least 12h before the experiment, full medium was exchanged for serum starved-medium (containing 1% FBS instead of 10%). HMECs were treated with 25 U/ml of murine interferon-γ (IFNγ) alone for 8 hours and IFNγ (purchased from Merck) treatment was followed separately by treatment with 50 U/ml of Lipopolysaccharides (LPS)(provided by Sigma-Aldrich) for additional 4 hours and at the end LPS alone for 4 hours to induce signal integration pathway between IFNs and toll-like receptors (TLRs). Total DNA was isolated using GeneMATRIX Universal DNA Purification Kit (EURx, Gdansk, Poland). The expression levels were compared to reference genes Actin (Actb) for both Vascular smooth muscle cells (VSMCs) and human microvascular epithelial cells (HMEC).

Real-time PCR analyses were compared for both HMECs and SMCs and certain treatment conditions were considered as an important factor. All of the conducted PCR results were evaluated and discussed in order to choose the most significant expression levels and genes. Among the genes have selected, Cxcl10 showed significant up-regulation for the patient material samples from ischemic heart disease in comparison to control subjects. This gene is expressed in both human and mouse atherosclerotic

plaques. Cxcl10 plays a fundamental role as an anti-inflammatory factor and induces foam cell formation. To evaluate this gene in further studies, bigger group of samples are needed with the different population profiles.

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