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# Investigation of the role of TLR4 agonist in M1 and M2 macrophage polarization and its immunotherapeutic effects in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most important causes of cancer-related deaths. The availability and efficacy of treatment options for HCC patients are limited. Therefore, the number of immunotherapy studies for the treatment of advanced HCC is increasing day by day. The aim of this study was to investigate the effects of soluble factors obtained from HCC cell culture stimulated with TLR4 agonist on macrophage polarization and to reveal new immunotherapeutic targets that can be used in the treatment of HCC. Supernatants obtained from TLR4 agonist (Lipopolisakkarit, LPS) stimulated HuH7 cells and control groups were fed to THP1 cell lines and the direction of macrophage polarization was examined. The expression of NOS2 gene for M1 macrophages and ARG1 gene for M2 macrophages were analyzed by RT-PCR. The results obtained were evaluated by statistical analysis. When THP1 Control and THP1+HuH7 Supernatant groups were compared, it was found that ARG1 expression was statistically significantly increased while NOS2 expression was statistically significantly decreased in HuH7 Supernatant added THP1 cells. In THP1 cells supplemented with LPS-induced HuH7 Supernatant (THP1 HuH7+LPS Supernatant), ARG1 and NOS2 expression was significantly increased. We found that LPS stimulates TLR4, allowing the tumor environment to change and transform into M1 macrophages, the lethal form of macrophages. Increased expression of NOS2 in macrophages may indicate increased tumor lethality and decreased tumor-induced immunosuppressive effect. We think that TLR4 is a good mediator for targeted therapies.

**Keywords:** HuH7 cells, THP1 cells, Lipopolysaccharide, Tumor microenvironment

## Introduction