Esophageal squamous cell carcinoma (ESCC) is the dominant histological type among esophageal cancers and ranked as the sixth leading cause of cancer death worldwide. ESCC has been identified as a very aggressive, fast growing and metastasizing cancer. Despite recent improvements in diagnostics and therapy, the 5-year survival rate for ESCC patients is low and is estimated at about 20%. Therefore, there is a need to elucidate the molecular mechanisms underlying this disease. Besides alcohol and tobacco, chronic inflammation has been identified as an important risk factor for ESCC. Several pro-inflammatory cytokines, such as TNF-α, secreted by cells of immune system have been shown to be upregulated in patients with ESCC, suggesting their important role in the tumor development. TNF-α is a well-known cytokine with pleiotropic actions that can act as a tumor-suppressing and tumor-promoting factor. TNF-α is a well-known stimulator of the expression of many genes known to promote the invasion and metastasis, such as MMP-9, by activation of the transcription factors, including NF-κB, AP-1 or SP-1. MMP9 is a zinc- and calcium-dependent endopeptidase and belongs to the main mediators of the degradation of ECM proteins. MMP9 can also selectively degrade non-matrix substrates such as chemokines, growth factors or integrins, and thereby contributes to the regulation of signaling events. Thus, MMP-9 is regarded to play a prominent role in the tumor growth. Over 60% of ESCC have been characterized by the overexpression of MMP9. Our preliminary data have shown direct associations between the TNF-α-induced MMP9 gene expression and CDKN1A/p21$^{(WAF1/CIP1/SDI1)}$, indicating an unknown function of CDKN1A as a regulator of proteolytic activity in cancer cells. CDKN1A protein, known as a cyclin-dependent kinase (CDK) inhibitor, plays important roles in regulation of DNA replication and transcription. It can both repress and induce various genes which are broadly known to be implicated in cancer growth. As an intrinsically disordered protein CDKN1A adopts an extended conformation and may be able to bind to proteins and DNA. Considering the increasing role of CDKN1A in human cancer, it seems that there is a need for a thorough analysis of the CDKN1A-MMP9 relationship in a context of the inflammatory response and the role of the immune system, as main sources of pro-inflammatory cytokines, in growth and metastasis of squamous cell carcinoma.

We propose here to characterize the mechanism of the CDKN1A activation and its role in regulation of TNF-α-induced MMP9 gene expression in relation to the promotion of invasion and metastasis in ESCC.

The specific aims of this research proposal are:

1) To determine whether CDKN1A is a transcription factor for MMP9 gene, acts synergistically with other transcription factors (AP-1, SP-1 and NFκB) known to activate the MMP9 gene promoter, or not.
2) To determine whether the regulation of MMP9 gene expression by CDKN1A depends on NFκB and whether these pathways are alternative, or parallel.
3) To answer the question whether the TNF-α-induced activation of CDKN1A gene expression in cancer cells is determined by certain pattern of cell-surface receptors for TNF-α, or not. If so, which of them (TNFR1 or TNFR2) is dominant.
4) To identify the potential correlation of CDKN1A and MMP9 expression in ESCC tissues and to determine its association with the immune cell infiltration of tumor microenvironment in relation to the level of TNF-α.

An identification of the precise mechanism of the regulation of TNF-α-induced MMP-9 activation by CDKN1A is a necessary step in better understanding the role of CDKN1A in relation to the tumor microenvironment immunity in the promotion of cancer cell survival and migration. It can help to define precisely the circumstances determining the role of the immune system as a friend or an enemy in the war on cancer.