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Institutet**

Department Of Microbiology, Tumor and Cell Biology

THE ROLE OF EBNA BINDING PROTEINS IN CELL TRANSFORMATION

AKADEMISK AVHANDLING

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av

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ABSTRACT

Epstein-Barr virus (EBV) infects majority of the human population and maintains sub-clinical infection. However, under certain conditions it is associated with several B-cell malignancies, such as Burkitt lymphoma, Hodgkin's lymphoma etc. Moreover, EBV also plays a causative role in acquired immunodeficiency syndrome (AIDS) associated lymphomas and post-transplant lymphoproliferative disease (PTLD). EBV maintains latent infection and expresses a particular set of proteins that are necessary for host cell proliferation. Studying function of EBV latent proteins could help us to understand the mechanisms underlying EBV induced B-cell transformation.

EBV transformed B cells, i.e. lymphoblastoid cell lines (LCLs) is a well-established *in vitro* model system to study the molecular mechanisms of B-cell transformation. In the present work, we have identified vitamin D receptor (VDR) as a binding partner of EBNA3. We showed that EBNA3 can block the VDR mediated gene transactivation and protects B-cells from vitamin D3 induced growth arrest/ apoptosis. We have observed that hypoxia inducible factor 1 alpha (HIF1 α) is stabilized in LCLs at normoxic conditions. HIF1 α is not hydroxylated and therefore it is not degraded in LCLs. We have shown that prolylhydroxylases 1 and 2 (PHD1 and 2) that are responsible for hydroxylation of HIF1 α , form complexes with EBNA5 and EBNA3, respectively. Due to this binding catalytic activity of PHDs is blocked, resulting in inhibition of HIF1 α hydroxylation and subsequent degradation. Stabilized HIF1 α is transcriptionally active and induces genes that are involved in glycolysis. Moreover, LCLs have high levels of pyruvate and lactate in contrast to mitogen activated B cells, indicating induction of aerobic glycolysis or Warburg effect.

We have shown that mitochondrial ribosomal protein MRPS18-2 (S18-2), an EBNA6 binding protein, can immortalize rat embryonic fibroblasts (REFs). These immortalized cells express stem cell markers like SSEA1, Sox2, Oct3/4 and have the characteristics of embryonic stem cells. S18-2 also immortalized the adult rat skin fibroblasts (RSFs). Moreover, single clones from immortalized REFs and RSFs resulted in tumors in SCID mice.

This thesis work reveals three different aspects of EBV induced B-cell transformation, i.e. protection from vitamin D3 induced apoptosis, metabolic adaptation required for proliferation and hijacking functions of novel protein MRPS18-2 for immortalization.