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Latent membrane protein 1 encoded by Epstein-Barr virus modulates directly and synchronously cyclin D1 and p16 by newly forming a c-Jun/Jun B heterodimer in nasopharyngeal carcinoma cell line

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Abstract

Recently we confirmed that latent membrane protein 1 (LMP1) encoded by Epstein-Barr virus (EBV) accelerates a newly forming active c-Jun/Jun B heterodimer, a transcription factor, but little is known about the target gene regulated by it. In this paper, results indicated that a c-Jun/Jun B heterodimer induced by LMP1 upregulated cyclin D1 promoters activity and expression, on the contrary, downregulated p16, and maladjustment of cyclin D1 and p16 expression accelerated progression of cell cycle. Firstly, we found a c-Jun/Jun B heterodimer regulated synchronously and directly cyclin D1 and p16 in the Tet-on-LMP1-HNE2 cell line, in which LMP1 expression is regulated by Tet-on system. This paper investigated in depth function of the newly forming active c-Jun/Jun B heterodimer, and built new connection between environmental pathogenic factor, signal transduction and cell cycle.

Keywords: Latent membrane protein 1; Nasopharyngeal carcinoma; Epstein-Barr virus; Activator protein 1; Heterodimer; c-Jun; Jun B; p16; Cyclin D1; Cell cycle

1. Introduction

EBV is a ubiquitous human herpesvirus that infects over 90% population of the world. LMP1, which can transform rodent fibroblasts and make nude mice tumorigenic, is considered to be a major oncogenic protein encoded by EBV (Eliopoulos and Young, 1998). LMP1 is an integral membrane protein composed of a short cytoplasmic N-terminus of 24 amino acids, six transmembrane domains of 162 amino acids and a cytoplasmic C-terminus of 200 amino acids. The carboxyl terminus comprises two functional domains: the membrane proximal c-terminal activation region-1 (CTAR1) and the membrane distal CTAR2 (residues 351–386). LMP1 can activate AP1 signal pathway through CTAR2 fuction region, thus causing abnormal cell proliferation and phenotypic changes (Vogt, 2001).

Recent findings show that roles of c-Jun or Jun B, important components of AP1 family, are complex, depending on the cellular context and cell type (Deng and Karin, 1993; Mathas et al., 2002; Leaner et al., 2003; Szabowski et al., 2000). c-Jun overexpression makes tumors invasive (Wisdom et al., 1999; Smith et al., 1999), on the other hand, also, triggers apoptosis (Bossy-Wetzel et al., 1997; Wang et al., 1999a). Jun B is always deemed to the inhibitory component of AP1 transcription factor (Passegue et al., 2001),

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and it can regulate erythroid and myogenic differentiation (Jacobs-Helber et al., 2002; Chalaux et al., 1998), however, some findings demonstrate that Jun B promotes carcinogenesis (Mao et al., 2003; Robinson et al., 2001).

Further study indicates that Jun B is a negative regulator of c-Jun (Szabowski et al., 2000; Chiu et al., 1989), and Jun B represses c-Jun by forming an inactive heterodimer (Deng et al., 2003). The balance of Jun B with c-Jun activity is involved in regulating the key steps in the proliferation and differentiation process (Schlingensiepen et al., 1993). However, some findings show that c-Jun and Jun B cooperatively regulate some target genes in an AP1-dependant manner, which promotes the cell proliferation and differentiation abnormally (Mathas et al., 2002; Leaner et al., 2003).

Our study demonstrated in Tet-on-LMP1-HNE2 cell line, LMP1 triggered a signaling cascade via its CTAR2 binding TRADD/TRAF2 signaling molecular, activated JNK and then upregulated AP1 activity including c-Jun and c-fos (Liao et al., 1999a,b; Deng et al., 2003; Wang et al., 2002). Using Atlas^{TM} Apoptosis cDNA Expression Array, we found that LMP1 induced both Jun B and c-Jun in the time- and dose-dependent manner in Tet-on-LMP1 HNE2 cell line (data not shown).

More recently, we have proved that LMP1 activates c-Jun NH2-terminal kinase (JNK), regulates protein expression and post-translational modification of Jun B and c-Jun and then induces an active c-Jun/Jun B heterodimer formation (Song et al., 2004). To further study the function of the c-Jun/Jun B heterodimer, it is crucial to find target gene regulated by it.

To be important, recently, a new direct connection was found between c-Jun and cyclin D1 related to G1/S checkpoint of cell cycle. c-Jun activated cyclin D1 promoters activity by binding to two AP1-alike sites in cyclin D1 promoter region in the HeLa cell line (Herber et al., 1994). Jun B and c-Jun were a pair of antagonistic transcriptional modulator, and the ratio of Jun B/c-Jun determined cyclin D1 transcription in the early G1 phage and balance of cell cycle. Phosphorylation of Jun B and c-Jun strengthened their transcriptional activity, regulated their dynamic balance, and at last modulated cyclin D1 promoters activity, influencing cyclin D1 expression (Bakiri et al., 2000).

In the meantime, also, a new direct connection is recently found between Jun B and p16, a negative modulator related to cell cycle. Three AP1-like binding sites were identified in the mice p16 promoter region. Jun B can activate directly p16 transcription through binding to these sites (Passegue and Wagner, 2000). Using Domain Architecture Retrieval Tool (http://www.ncbi.nlm.nih.gov/Structure/Dart/Lexington/html/overview.html), we found that Jun B domains between human and mice are highly homologous (99%), and there is only an amino acid difference in the basic leucine zipper domain (bZIP) of Jun B. We further predicted that four putative AP1 bind sites exist in the human p16 promoters using bioinformatics (http://www.cbrc.jp/research/db/TFSEARCH.html).

Using Atlas^{TM} human cDNA expression array I (Clontech) detecting normal human nasopharyngeal tissue and nasopharyngeal carcinoma, we found cyclin D1 overexpression in the nasopharyngeal carcinoma at the transcriptional level (Li et al., 1998; Xie et al., 2000). In the meantime, we proved cyclin D1 protein expression increased gradually in the multistage tissue of the simple hyperplasia/metaplasia, atypical hyperplasia/metaplasia and nasopharyngeal carcinoma during nasopharyngeal carcinogenesis, strengthening significantly especially in the atypical hyperplasia/metaplasia tissue (data not shown). Further study indicated LMP1 encoded by EBV regulated cyclin D1, thus led to disorder of proliferation and maladjustment of apoptosis (Zhao et al., 2000, 2001).

On the other hand, point mutation of p16 (Sun et al., 1995) occurs rarely in the human nasopharyngeal carcinomas, but there exists frequent inactivation of p16 (Huang et al., 2001; Baba et al., 2001), or lack (Gulley et al., 1998; Makitie et al., 2003; Lo et al., 1995), or hypermethylation of its promoters (Jefferies et al., 2001; Chang et al., 2003; Lo et al., 1996). Restoration of p16 in nasopharyngeal carcinoma inhibited significantly tumorigenic potential (Wang et al., 1999b; Lee et al., 2003). Meanwhile, EBV inhibited p16 expression in human fetal nasopharyngeal epithelial cells escaping from the replicative senenscence (Yang et al., 2003).

These studies above all suggest that cyclin D1 and p16 may be adjusted synchronously by the c-Jun/Jun B heterodimer induced by LMP1.

So, based on progress on study of literature and our work done (Song et al., 2004), in this paper, we want to investigate in depth whether a c-Jun/Jun B heterodimer induced by LMP1 could regulate cyclin D1 and p16 synchronously, which are closely related to cell cycle, and accelerate cell cycle progression.

2. Materials and methods

2.1. Cell culture

HNE2 is EBV-negative, poorly differentiated NPC cell line. Tet-on-LMP1-HNE2 was constructed by transfecting LMP1 expressin plasmid and tet-on system into HNE2. In the Tet-on-LMP1-HNE2, LMP1 and tet-on was stably integrated, the expression of LMP1 will be turned on by tetracycline (Doxycline, Dox). Two cell lines were established by Cancer Research Institute, Xiangya School of Medicine, Central South University (Liao et al., 1999a,b, 2001). Tet-on-LMP1-HNE2 cells were cultured in RPMI 1640 medium (GIBCO BRL) containing 100 μg/ml G418, 50 μg/ml hygromycin and 10% FCS (GIBCO BRL) in a humidified 5% CO2 atmosphere at 37 °C. To induce LMP1 expression, cells were treated with Dox at 0, 0.006, 0.06, 0.6 and 6.0 μg/ml, respectively. In the meanwhile, with 0.6 μg/ml Dox induction, cell was collected at the time points of 0, 0.5, 1, 2, 4, 8, 12, 18 and 24 h.