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Adenovirus E1A TRRAP-targeting domain-mediated enhancement of MYC association with the NuA4 complex activates a panel of MYC target genes enriched for gene expression and ribosome biogenesis

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ABSTRACT

Cellular transformation by adenovirus E1A requires targeting TRRAP, a scaffold protein which helps assemble histone acetyltransferase complexes, including the NuA4 complex. We recently reported that E1A and E1A 1–80 (N-terminal 80 aa) promote association of the proto-oncogene product MYC with the NuA4 complex. The E1A N-terminal TRRAP-targeting (ET) domain is required for E1A 1–80 to interact with the NuA4 complex. We demonstrate that an ET-MYC fusion associates with the NuA4 complex more efficiently than does MYC alone. Because MYC regulates genes for multiple cellular pathways, we performed global RNA-sequence analysis of cells expressing MYC or ET-MYC, and identified a panel of genes (262) preferentially activated by ET-MYC and significantly enriched in genes involved in gene expression and ribosome biogenesis, suggesting that E1A enhances MYC association with the NuA4 complex to activate a set of MYC target genes likely involved in cellular proliferation and cellular transformation by E1A and by MYC.

1. Introduction

Adenovirus E1A 243R (E1A) is a viral oncoprotein that interacts with several key host factors critical for E1A-mediated cellular transformation, including p300, Rb, and TRRAP (for review see (Berk, 2005; Pelka et al., 2008)). Targeting of Rb by E1A leads to E2F activation of genes important for G1/S transition in the cell cycle. E1A targeting of p300 causes global reduction in histone H3K18 acetylation (Horwitz et al., 2008), and repression of specific cellular and viral promoters (Green et al., 2008; Zhao et al., 2015, 2016b). While TRRAP targeting is essential for cellular transformation by E1A (Deleu et al., 2001; Fuchs et al., 2001), its function remains unclear. We recently demonstrated that E1A and E1A 1–80 (the N-terminal 80 aa) enhanced association of the proto-oncogene product MYC with the NuA4 histone acetyltransferase (HAT) complex, and that the E1A TRRAP-targeting (ET) domain is essential for E1A 1–80 to interact with the NuA4 complex (Zhao et al., 2016a). Significantly, E1A 1–80 specifically targets TRRAP that is present in the NuA4 complex and not TRRAP in other HAT complexes (Zhao et al., 2016a). Since an association with TRRAP is essential for transformation by E1A and by MYC (Deleu et al., 2001; McMahon et al., 1998; Park et al., 2001), it seems likely that enhanced MYC association with the NuA4 complex is involved in cellular transformation by E1A and by MYC.

MYC is a proto-oncogene overexpressed in many cancers and required by essentially all cancers (Dang, 2012; Meyer and Penn, 2008; Tansey, 2014). It is a transcription factor that forms a leucine-zipper with MAX to bind promoters and activate genes of multiple cellular pathways that govern cellular proliferation (Blackwood and Eisenman, 1991). It has been estimated that up to 15% of cellular gene promoters can be bound by MYC (Patel et al., 2004; Zeller et al., 2006). Importantly, only a small portion of these are activated by over-expressed MYC (Fernandez et al., 2003), suggesting that factors in addition to promoter binding may determine whether the MYC-bound promoter is activated. HAT complexes, including the NuA4 complex and the GCN5 complex, can be recruited by MYC to gene promoters for transcriptional activation (Frank et al., 2003; Liu et al., 2003; McMahon et al., 2000). The specific roles of HAT complexes during cellular transformation and cancer development remain poorly understood.

The human NuA4 complex is involved in chromatin remodeling, gene activation, and DNA damage repair (Doyon et al., 2004; Lee and Workman, 2007; Squatrito et al., 2006). It has a core HAT enzyme Tip60, and is one of the largest HAT complexes containing up to 20 subunits (Lee and Workman, 2007). It acetylates primarily histones H2

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and H4, and is believed to represent a fusion complex of the yeast chromatin remodeling complex SWR1 and the yeast NuA4 complex (Auger et al., 2008; Doyon et al., 2004). MYC and several components of the NuA4 complex co-occupy promoters of certain genes that are highly expressed in mouse embryonic stem cells (Kim et al., 2010). Significantly, over-expression of a similar set of genes is also detected in human cancers, suggesting the importance of the MYC-NuA4 pathway in cancer (Kim et al., 2010).

We propose that E1A enhances MYC association with the NuA4 complex to specifically activate a subset of MYC target genes that facilitate cellular transformation. To examine this hypothesis, we performed a global analysis of gene regulation by MYC association with the NuA4 complex and identified a panel of 262 genes activated by efficient MYC association with the NuA4 complex.

2. Results

2.1. E1A TRRAP-targeting (ET) domain fused to the MYC N-terminus enhances MYC association with the NuA4 complex

It was shown that an E1A N-terminal fragment (aa 12–54) which interacts with TRRAP can substitute for the N-terminal region of MYC (aa 1–262) for transformation activity of MYC (Deleu et al., 2001), suggesting that the E1A N-terminal domain can function independently of other E1A sequences, and that MYC N-terminal fusion constructs can be functionally active. We previously reported that E1A 1–80 interacts with the NuA4 complex through the ET domain, thereby enhancing association of MYC with the NuA4 complex (Zhao et al., 2016a). We surmised that the ET domain fused to MYC could also enhance MYC association with the NuA4 complex, resulting in a sensitive probe for analyzing gene regulation by increased MYC association with the NuA4 complex in a biologically relevant manner. This construct would mimick the enhanced MYC-NuA4 complex promoted by E1A and E1A 1–80 (Zhao et al., 2016a).

The ET domain (defined here as E1A aa 11–62) together with a Flag-tag was fused to the MYC N-terminus to generate an ET-MYC fusion protein that is expressed from a lentivirus vector (Fig. 1A). ED-MYC, used as a negative control, has a deletion of E1A aa 26–35, which are important for E1A 1–80 interaction with TRRAP and the NuA4 complex (Zhao et al., 2016a). To initially examine their interaction with the NuA4 complex, ET-MYC and ED-MYC were expressed in HeLa cells. FH-MYC (with an N-terminal Flag-HA tag) was used as an additional control. Co-immunoprecipitation of the cell lysates with Flag antibody followed by Western blot analysis reveals that, as expected from our previous results (Zhao et al., 2016a), FH-MYC binding to NuA4 components is inefficient (Fig. 1B, lane 1). In contrast, ET-MYC strongly binds to all of the NuA4 components examined (lane 2), while ED-MYC has a greatly reduced ability to interact with the NuA4 complex (lane 3). Interaction of ED-MYC with the NuA4 components (lane 3) is still more efficient than that of FH-MYC (lane 1), possibly due to residual TRRAP-targeting activity of the mutated ET domain.

MYC associates with Max to form a leucine-zipper heterodimer (Blackwood and Eisenman, 1991), which is responsible for high-affinity binding of MYC/Max to the E-box elements on gene promoters (Zeller et al., 2006). Western blot analysis of the Flag immunoprecipitates with Max antibody showed that FH-MYC, ET-MYC and ED-MYC associates with Max to similar levels (Fig. 1B), suggesting that the association of MYC with Max is unaffected by the addition of the ET domain.

TRRAP interaction with E1A and MYC has been mapped to two regions of TRRAP (Deleu et al., 2001; Park et al., 2001). It is possible that the ET domain interacts efficiently with TRRAP in the NuA4 complex, and facilitates MYC association with a separate region of TRRAP, possibly through conformational changes in TRRAP induced by the interaction with the ET domain (Fig. 1C). The ET-MYC-NuA4 complex is expected to functionally resemble the MYC-NuA4 complex promoted by E1A and E1A 1–80 (Zhao et al., 2016a).

2.2. ET-MYC activates selected MYC target genes in human fibroblasts

Since over-expression of MYC is toxic to cells (Shachaf et al., 2008), to examine potential gene regulation by ET-MYC, we constructed inducible lentiviral expression vectors for MYC (without epitope tag) and ET-MYC in the pLVX-TetOne-puro vector (Fig. 2A) (see “Materials and Methods”). The pLVX-TetOne-puro vector simultaneously expresses the Tet-On activator, so that expression of the gene of interest is induced by inclusion of doxycycline in the cell culture medium. To avoid potential toxicity and secondary effects due to MYC over-expression, we induced expression of MYC and ET-MYC for 18 h under a sub-optimal induction condition with doxycycline (60 ng/ml versus the usual 100 ng/ml).

Despite the large number of gene promoters bound by MYC, only a small fraction of these genes have been shown to be specifically activated by over-expressed MYC (Fernandez et al., 2003). Further, endogenous MYC target genes are normally expressed well in proliferating cancer cells that are commonly used in MYC studies. Consequently, we used HS68 cells (human foreskin fibroblasts) which are normal in many respects, including MYC expression level. In culture, HS68 cells become contact-inhibited after forming a confluent monolayer, presumably resulting in down-regulation of genes important for cell proliferation. However, they resume growth and proliferation after re-plating, and thus are suitable for analysis of gene regulation by MYC.

To examine gene regulation by ET-MYC in HS68 cells, we transduced cells with the inducible expression lentiviruses for MYC and ET-MYC, or the lentivirus expressing luciferase as control. After puromycin selection, cells were allowed to grow to confluence and become contact-inhibited. Cells were then re-plated and expression of ET-MYC or MYC from the vector was induced with doxycycline. Cell RNA was prepared and RT-qPCR analysis performed with gene-specific primers for selected MYC target genes: NCL (nucleolin) and MCT1 (monocarboxylate transporter 1) which are over-expressed in certain cancers (Hong et al., 2016; Otake et al., 2007), and NOP2 (nucleolar protein 2 homologue) (Kim et al., 2010). As shown in Fig. 2B, all the three genes are activated by MYC and ET-MYC to similar levels (see also Table S2). In contrast, CD44, which is not known to be a MYC target gene and is expressed at high levels in breast cancer cells (Kim et al., 2016), is not activated by MYC or ET-MYC. Since MYC over-expression is correlated with p53 induction (Shachaf et al., 2008), we also examined p53 expression by RT-qPCR and found that both MYC and ET-MYC activate p53 (Fig. 2B). From these results, we conclude that ET-MYC is functionally comparable to MYC. As shown in Fig. 2C, induced ET-MYC mRNA is ~60% of that of MYC. Thus, ET-MYC activation of NCL, MCT1 and NOP2 (Fig. 2B) is likely more efficient than activation by MYC, despite the apparently similar levels of activation by MYC and ET-MYC.

2.3. Global gene expression analysis and identification of a panel of genes preferentially activated by MYC association with the NuA4 complex

Transcriptional regulation by MYC normally involves transcription regulators in addition to the NuA4 complex, such as the GCN5 complex (Liu et al., 2003; McMahon et al., 2000) and the elongation factor PAF1C (Jaenicke et al., 2016). MYC association with the NuA4 complex is normally weak (Kim et al., 2010) (see Fig. 1). However, E1A and E1A 1–80 greatly enhance MYC association with the NuA4 complex (Zhao et al., 2016a). The efficient association of ET-MYC with the NuA4 complex (Fig. 1) suggests that ET-MYC will preferentially up-regulate MYC target genes that are normally activated by MYC association with the NuA4 complex. Since the ET domain is essential for E1A-mediated