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GADD45a physically and functionally interacts with TET1

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ABSTRACT

DNA demethylation plays a central role during development and in adult physiology. Different mechanisms of active DNA demethylation have been established. For example, Growth Arrest and DNA Damage 45-(GADD45) and Ten-Eleven-Translocation (TET) proteins act in active DNA demethylation but their functional relationship is unresolved. Here we show that GADD45a physically interacts – and functionally cooperates with TET1 in methylcytosine (mC) processing. In reporter demethylation GADD45a requires endogenous TET1 and conversely TET1 requires GADD45a. On GADD45a target genes TET1 hyperinduces 5-hydroxymethylcytosine (hmC) in the presence of GADD45a, while 5-formyl-(fC) and 5-carboxylcytosine (caC) are reduced. Likewise, in global analysis GADD45a positively regulates TET1 mediated mC oxidation and enhances fC/caC removal. Our data suggest a dual function of GADD45a in oxidative DNA demethylation, to promote directly or indirectly TET1 activity and to enhance subsequent fC/caC removal.

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1. Introduction

DNA methylation at the C5 position of cytosine (mC) is a well-characterized epigenetic mark in higher eukaryotes (reviewed in Bird (2002), Deaton and Bird (2011), Hackett and Surani (2013) and Jones and Takai (2001)). mC typically confers transcriptional silencing within gene regulatory regions and propagates this silenced state to daughter cells. DNA methylation can be very dynamic *e.g.* during epigenetic reprogramming, early embryonic development and cellular differentiation (reviewed in Messerschmidt et al. (2014), Niehrs (2009), Williams et al. (2012) and Wu and Zhang (2010)). During these phases active DNA demethylation, the enzymatic removal of mC, is crucial to shape the epigenetic signature in order to activate key developmental genes (reviewed in Guo et al. (2011a), Messerschmidt et al. (2014), Niehrs and Schäfer (2012), Pastor et al. (2013), Schäfer (2013) and Wu and Zhang (2010)). In animals, three main mechanisms of active DNA demethylation have been proposed: DNA demethylation (i) by nucleotide-excision repair (NER; Barreto et al., 2007), (ii) by base-excision repair (BER) upon mC deamination by AID (Activation Induced Deaminase; Cortellino et al., 2011; Morgan et al., 2004), and (iii) by mC oxidation mediated by the Ten-Eleven Translocation (TET) family enzymes followed by BER (Maiti and Drohat, 2011; Shen et al., 2013; Tahiliani et al., 2009).

A regulatory protein family in NER- and BER-based DNA demethylation is GADD45 (Growth Arrest and DNA Damage Protein 45a,-b,-g). GADD45 proteins are devoid of any obvious enzymatic activity and act as adapters between demethylation target genes and the DNA repair machinery. For example, GADD45a binds to distinct genomic loci *via* the H3K4me3 reader ING1b (Schäfer et al., 2013), the RNA polymerase cofactor TAF12 (Schmitz et al., 2009), or the lncRNA TARID (Arab et al., 2014) to recruit DNA repair enzymes such as the 3′-NER endonuclease XPG (Barreto et al., 2007; Le May et al., 2010; Schmitz et al., 2009), the BER enzyme Thymine DNA Glycosylase TDG (Arab et al., 2014; Cortellino et al., 2011; Li et al., 2015), and AID (Cortellino et al., 2011; Rai et al., 2008).

An important question is whether GADD45 also interacts with TET-mediated, oxidative DNA demethylation. TET dioxygenases iteratively oxidize the methyl group at C5 to yield 5-hydroxymethyl-(hmC) (Kriaucionis and Heintz, 2009; Tahiliani et al.,

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2009), 5-formyl-(fC) (Maiti and Drohat, 2011) and 5-carboxylcytosine (caC) (He et al., 2011; Maiti and Drohat, 2011). caC can be decarboxylated by bacterial and mammalian C5-DNA methyltransferases *in vitro* (Liutkeviciute et al., 2014). *In vivo* however, only TDG mediated excision of fC and caC has been shown to accomplish DNA demethylation. The resulting abasic site is processed by BER to incorporate unmethylated C (Cortellino et al., 2011; He et al., 2011; Maiti and Drohat, 2011). Recently it has been shown that GADD45a enhances TDG mediated removal of fC and caC (Li et al., 2015). Thus, TDG is a common component of both, TET- and GADD45 mediated DNA demethylation. Together with the finding that GADD45a and TDG are required for TET mediated demethylation of *TCF21* (Arab et al., 2014) this raises the question, if Gadd45a may directly interact with TET enzymes.

Here we show that GADD45a and TET1 directly bind each other. Moreover, GADD45a positively regulates TET1 induced mC oxidation and the two proteins require each other for reporter demethylation. Furthermore, GADD45a reduces fC and caC levels, both gene-specifically as well as globally. Our data corroborate a close link between the GADD45a- and TET1-mediated DNA demethylation pathways.

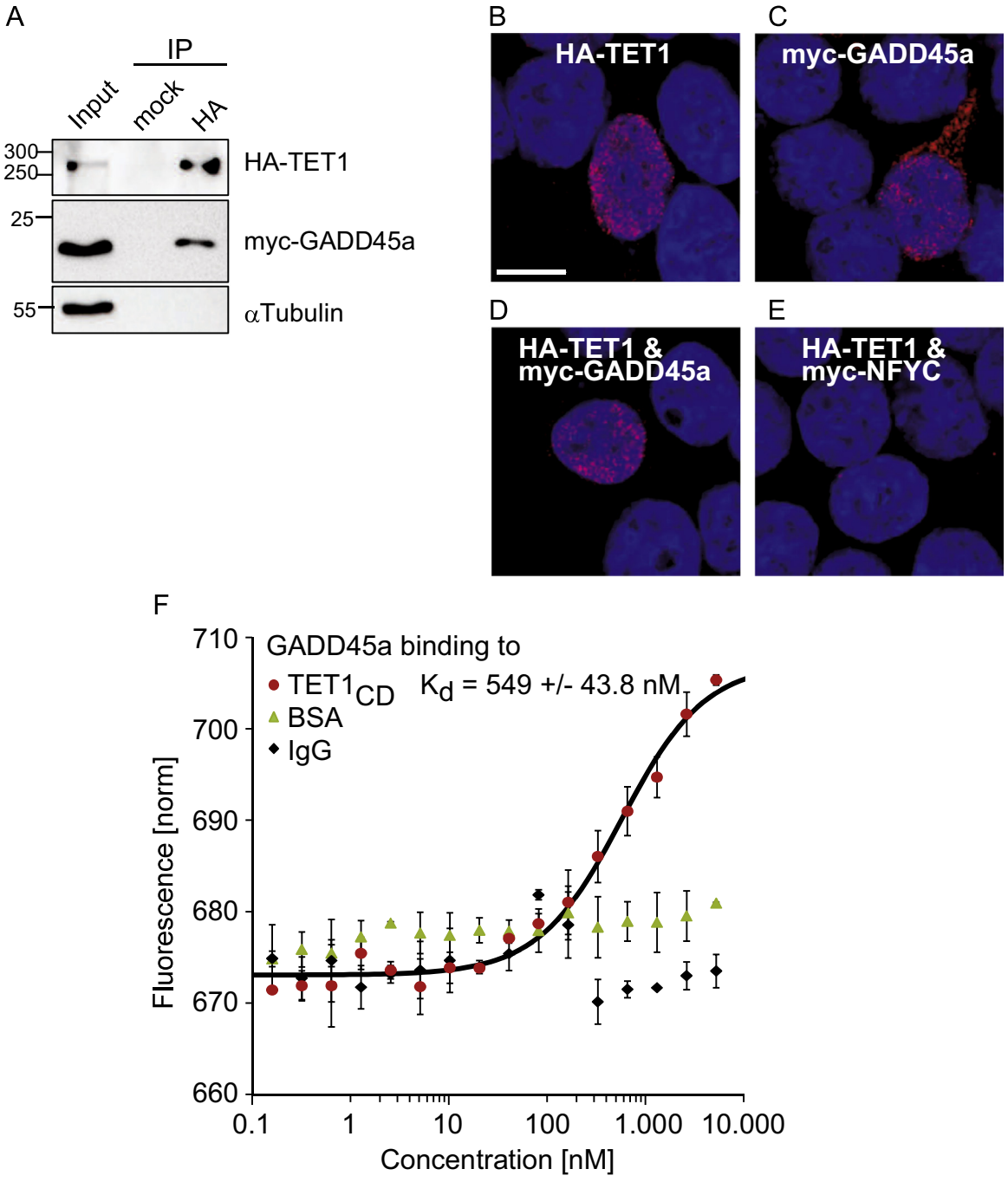

Fig. 1. GADD45a directly binds TET1. (A) Western blot analysis of co-immunoprecipitation (Co-IP) experiments using protein lysates of HEK293T cells transfected with Flag-HA-TET1 and myc-GADD45a. α-Tubulin was used as specificity control. Input shows 5% of lysate used for IP. (B–E) *In-situ* analysis of TET1-GADD45a interaction by Proximity Ligation Assay (PLA) in HEK293T cells transiently transfected as indicated. Scale bar, 10 μm. (F) Microscale Thermophoresis binding assays of fluorescently labeled recombinant GADD45a to $TET1_{CD}$ or the negative controls BSA and IgG. Concentration of GADD45a was kept constant at 50 nM, while increasing concentrations of $TET1_{CD}$, BSA and IgG were tested (0.1 nM to 5 μM). Each measurement point represents the mean of biological replicates ($n=3$) with error bars as ± SD.

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