HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2015 January 20.

Published in final edited form as:

J Invest Dermatol. 2014 May ; 134(5): 1470–1472. doi:10.1038/jid.2013.489.

Tumor Necrosis Factor-Alpha and Apoptosis Induction in Melanoma Cells through Histone Modification by 3-Deazaneplanocin A

Ryo Tanaka[1], **Nicholas Donovan**[1], **Qiang Yu**[3], **Reiko Irie**[2], and **Dave S. B. Hoon**[1]

[1]Department of Molecular Oncology, John Wayne Cancer Institute at Saint John's Health Center, Santa Monica, CA

[2]Biotechnology Science, John Wayne Cancer Institute at Saint John's Health Center, Santa Monica, CA

[3]Cancer Biology and Pharmacology, Genome Institute of Singapore, Singapore

Keywords

TNFα; DZNep; histone; melanoma; methylation

To the Editor,

Malignant melanoma is a highly aggressive skin cancer that can be difficult to manage once metastasis has occurred. Tumor necrosis factor-alpha(TNFα) is a cytokine that influences the tumor microenvironment, activates tumor inflammation, and induces cell death(Balkwill, 2009). TNFα induces the signal-transduction pathways associated with cell survival through NF-κB or caspase 8. Melanoma cells can produce TNFα(Landsberg *et al.*, 2012); however, its expression is heterogeneous and regulation of tumor cell TNFα production is poorly understood.

Epigenetic deregulation plays an important role in aberrant gene expression and melanoma progression(Tanemura *et al.*, 2009). Several tumor-related genes are consistently aberrantly hypermethylated during melanoma progression(Greenberg *et al.*, 2012; Hoshimoto *et al.*, 2012; Tanemura *et al.*, 2009). Similarly, histone modification has also been shown to regulate gene expression(Kouzarides, 2007) by affecting both the initiation and progression of cancer by various mechanisms. An important repressive histone mark H3K27me3 is induced by enhancer of zeste homologue 2(EZH2) (Chang and Hung, 2011).

Requests for reprints: Professor Dave S.B. Hoon, Dept of Molecular Oncology, John Wayne Cancer Institute at Saint John's Health Center, 2200 Santa Monica Blvd, Santa Monica, CA 90404; hoon@jwci.org.

Supplementary Material

Supplementary material is linked to the online version of the manuscript at http://www.nature.com/jid

Conflict of Interest

No authors declare conflict of interest.

3-Deazaneplanocin A(DZNep) is a potent S-adenosylhomocysteine hydrolase inhibitor which can indirectly inhibit S-adenosyl-methionine(AdoMet)–dependent reactions related with various methyltransferases(Miranda *et al.*, 2009). It has been shown to inhibit the histone methyltransferase Polycomb-repressive complex 2(PRC2) components EZH2, suppressor of zeste 12 homolog(SUZ12), and embryonic ectoderm development(EED)(Tan *et al.*, 2007). DZNep acts as an epigenetic modifying agent that represses H3K27me3(Tan *et al.*, 2007).

In breast cancer cells, but not normal cells, DZNep can induce apoptosis(Tan *et al.*, 2007). Trichostatin A(TSA), a histone deacetylase inhibitor, can block histone hypoacetylation to restore expression levels of several tumor suppressor genes and induce apoptosis in human cancer cells such as melanoma. Recently, we demonstrated that the combination DZNep and TSA can induce repression of H3K27me3 and elevation of H3K9ac in prostate cancer cells to significantly amplify tissue inhibitor of metalloproteinase-3(TIMP3) expression(Shinojima *et al.*, 2012).

To investigate the effect of histone modification to DZNep on melanoma cells, we treated and assessed five melanoma cell lines. Four of the five DZNep-treated cell lines (M12, M15, M101, and M223) resulted in repression of H3K27me3 and PRC2 components EZH2 and SUZ12. In addition, cleaved poly(ADP-ribose) polymerase(PARP) was detected, indicating that DZNep could induce apoptosis in these lines(Figure 1). Interestingly, H3K4me2 was repressed in the M15, M101, and M223 lines, demonstrating that DZNep could repress PRC2 and H3K27me3 in specific melanoma cell lines.

To determine if cell death was induced through apoptosis, two types of assays were performed on the M101 cell line which was highly sensitive to the DZNep treatment, using the optimal non-toxic dose(5μM) of DZNep based on a cell viability analysis. In propidium iodide (PI) staining and cell cycle analysis, the sub-G1 fraction was shown to be enhanced by DZNep. By flow cytometry analysis using annexin V-FITC and PI staining, DZNep exposure caused an increase in the percentage of cells gated for Annexin V+/PI−, early apoptosis and Annexin V+/PI+, and late apoptosis/necrosis(Supplemental Figure 1A-C). These results, combined with the western blot results, suggested that DZNep induced apoptosis in M101, M12, and M223 cell lines.

We proceeded to further identify apoptosis-specific gene expression induced by DZNep using RT–PCR-based RT^2 Profiler™ Human Apoptosis PCR array(Supplemental Table 1). mRNA expression of 10 genes was upregulated by ≥two-fold after DZNep treatment; TNFα mRNA was upregulated 6.3-fold. Therefore, we focused on TNFα expression.

We performed a ChIP-qPCR array using anti-H3K27me3 and anti-H3K4me2 Ab to identify DZNep-activated PRC2 target genes in melanoma cells. Fold enrichment by quantitative ChIP was calculated by the ratio to input DNA. Supplemental Table 2 shows 13 genes associated with decreased percentage input of repressive histone H3K27me3 and increased H3K4me2 in DZNep-treated cells compared to DMSO-treated cells. The percentage input on TNFα in H3K27me3 was decreased 2.0X while there was increase of 7.0X in H3K4me2.

To confirm the results of the TNFα ChIP-qPCR array, qRT-PCR was performed on M101, M12, LF0023, and M223. It was demonstrated that TNFα was upregulated in M101 and M223 cells by DZNep treatment(Supplemental Figure 2A, B, C). In 5-Aza treatment, induction of TNFα upregulation in M101, LF0023, and M223 cell lines was demonstrated. The M12 line was only upregulated by the combined treatment of both DZNep and TSA(Supplemental Figure 2B). For M12, H3K9ac was also strongly activated by TSA and DZNep, inducing TNFα(Supplemental Figure 3).

To confirm that TNFα was regulated by histone modification, we assessed the TNFα promoter region(−1536bp to +879bp of transcriptional start site) (Supplemental Figure 4). Using ChIP analysis, the percentage input ratio between H3K27me3 and H3 on TNFα in M101 was significantly decreased($p<0.01$) in DZNep-treated specimens compared to DMSO. For H3K4me2 and H3K9ac, there was no significant difference between DMSO and DZNep-treated cells(Supplemental Figure 5A). This trend in H3K27me3 was similar to results of the ChIP-PCR array(Supplemental Table 2).

To assess DNA methylation status of the TNFα promoter region, we performed a methylation-specific PCR assay(MSP). Methylated DNA in DMSO-treated cells was not changed by DZNep treatment(Supplemental Figure 5B). These results suggested that DZNep may affect the repressive histone H3K27me3, restoring TNFα expression level.

To assess whether DZNep modification of TNFα histone status was related to apoptosis, a ChIP analysis was performed on M12 and M223. For M12, the percentage input ratio between H3K27me3 and H3 on TNFα was not significantly decreased by DZNep and no DNA methylation was evident(Supplemental Figure 6A(i), B). The combined treatment of DZNep and TSA restored TNFα expression, suggesting that H3K9ac was related to the regulation of TNFα; however, H3K27me3 was not strongly associated with TNFα repression. In M223, the percentage input ratio between H3K27me3 and H3 on TNFα was significantly decreased by DZNep; DNA was also methylated(Supplemental Figure 6A(ii), B) and 5-Aza restored TNFα expression(Supplemental Figure 2B). These results suggested that DNA methylation and H3K27me3 play a significant role in TNFα regulation.

To assess the association between TNFα expression and epigenetic status in melanoma tissues, we performed RT-PCR, a ChIP-qPCR assay, and MSP(Supplemental Figure 7A-C) on melanoma tumors(n=4). The percentage input ratio between H3K27me3 and H3 was highest in PT4. TNFα could not be detected by qRT-PCR(Supplemental Figure 7A, B(i)). On the other hand, the percentage input ratio between H3K4me2 and H3, and the percentage input ratio between H3K9ac and H3 was highest in PT2, which also showed TNFα expression(Supplemental Figure 7B(ii, iii)). The analysis suggested that repressive and active histones were associated with TNFα regulation. In contrast, DNA methylation was not strongly related to the repression of TNFα expression(Supplemental Figure 7C). Among the four specimens, TNFα expression level was highest in PT1; however, the percentage input ratio between H3K27me3 and H3 was relatively high and the percentage input ratio between H3K4me2/H3K9ac and H3 was relatively low. In PT1, other mechanisms such as regulation of transcription, splicing, message turnover, or translation may play a dominant role in regulating TNFα expression over epigenetic factors.

In summary, we demonstrated that DZNep treatment induced apoptosis in melanoma cells, suggesting a change of chromatin architecture to a heterochromatin phenotype. We showed that DZNep treatment induced repression of PRC2 and H3K27me3, thereby restoring TNFα expression. In addition, we confirmed that H3K27me3 can bind to the promoter region of TNFα. Furthermore, the combination of DZNep and TSA restored TNFα expression, confirming a significant role of H3K9ac in TNFα upregulation. We showed that the TNFα promoter region was hypermethylated and mRNA expression of TNFα was restored by 5-Aza treatment. In combination, these results suggested that both DNA methylation and histone modification were strongly associated with the regulation of TNFα expression.

TNFα expression levels in melanoma lines are generally quite low. Previously, it was reported that repressive H3K27me3 was linked to *de novo* DNA methylation of PRC2 target genes(Schlesinger *et al.*, 2007). H3K9 histone methylation may also play an important role in DNA methylation(Ohm *et al.*, 2007). In contrast, it has been demonstrated that DNA methylation and repressive histone modification are independently correlated with silencing of various genes(Kondo *et al.*, 2008). Sullivan *et al.* reported the epigenetic regulation mechanism of TNFα both in development and acute stimulation in leukemia(Sullivan *et al.*, 2007). Although the expression level of H3K9ac in western blot analysis was unchanged, H3K4me2 and H3k9ac may be associated with the restoration of TNFα by DZNep treatment. In contrast, H3K27me3 was clearly repressed by DZNep. We confirmed that DZNep could release binding of H3K27me3 to the promoter region on TNFα and restore its expression. Since both 5-Aza and DZNep treatment alone activated TNFα expression, methylation of both the gene promoter and H3K27 may be independently associated with TNFα repression.

EZH2 expression in metastatic melanoma was shown to be significantly higher than *in situ* primary melanoma and benign nevi(McHugh *et al.*, 2007). Although DZNep may have other active mechanisms, its role in EZH2 inhibition may show potential for melanoma treatment. Indirect activation of TNFα in melanoma may be a promising alternative treatment approach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

This work was supported by the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation, Weil Family Foundation, the Leslie and Susan Gonda (Goldschmied) Foundation (Los Angeles, CA) and NIH, NCI P0 CA029605 Project II and Core C.

Abbreviation

Ab	antibody
DZNep	3-Deazaneplanocin A
DMSO	dimethyl sulfoxide

DNMTs	DNA methyltransferases
EED	embryonic ectoderm development
FCS	fetal calf serum
MSP	methylation-specific PCR assay
PBS	phosphate-buffered saline
PARP	poly (ADP-ribose) polymerase
PRC2	Polycomb Repressive Complex 2
PI	propidium Iodine
RIPA	radioimmunoprecipitation assay
AdoMet	S-adenosyl-methionine
SUZ12	suppressor of zeste 12 homologue (Drosophila)
TIMP3	tissue inhibitor of metalloproteinase-3
TSS	transcription start site
TSA	Trichostatin A

References

Balkwill F. Tumour necrosis factor and cancer. Nat Rev Cancer. 2009; 9:361–371. [PubMed: 19343034]

Chang CJ, Hung MC. The role of EZH2 in tumour progression. Br J Cancer. 2011; 106:243–247. [PubMed: 22187039]

Greenberg ES, Chong KK, Huynh KT, Tanaka R, Hoon DS. Epigenetic biomarkers in skin cancer. Cancer Lett. 2012 Epub Ahead of Print.

Hoshimoto S, Kuo CT, Chong KK, Takeshima TL, Takei Y, Li MW, et al. AIM1 and LINE-1 epigenetic aberrations in tumor and serum relate to melanoma progression and disease outcome. J Invest Dermatol. 2012; 132:1689–1697. [PubMed: 22402438]

Kondo Y, Shen L, Cheng AS, Ahmed S, Boumber Y, Charo C, et al. Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. Nat Genet. 2008; 40:741–750. [PubMed: 18488029]

Kouzarides T. Chromatin modifications and their function. Cell. 2007; 128:693–705. [PubMed: 17320507]

Landsberg J, Kohlmeyer J, Renn M, Bald T, Rogava M, Cron M, et al. Melanomas resist T-cell therapy through inflammation-induced reversible dedifferentiation. Nature. 2012; 490:412–416. [PubMed: 23051752]

McHugh JB, Fullen DR, Ma L, Kleer CG, Su LD. Expression of polycomb group protein EZH2 in nevi and melanoma. J Cutan Pathol. 2007; 34:597–600. [PubMed: 17640228]

Miranda TB, Cortez CC, Yoo CB, Liang G, Abe M, Kelly TK, et al. DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. Mol Cancer Ther. 2009; 8:1579–1588. [PubMed: 19509260]

Ohm JE, McGarvey KM, Yu X, Cheng L, Schuebel KE, Cope L, et al. A stem cell-like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing. Nat Genet. 2007; 39:237–242. [PubMed: 17211412]

Schlesinger Y, Straussman R, Keshet I, Farkash S, Hecht M, Zimmerman J, et al. Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. Nat Genet. 2007; 39:232–236. [PubMed: 17200670]

Shinojima T, Yu Q, Huang SK, Li M, Mizuno R, Liu ET, et al. Heterogeneous epigenetic regulation of TIMP3 in prostate cancer. Epigenetics. 2012; 7:1279–1289. [PubMed: 23023649]

Sullivan KE, Reddy AB, Dietzmann K, Suriano AR, Kocieda VP, Stewart M, et al. Epigenetic regulation of tumor necrosis factor alpha. Mol Cell Biol. 2007; 27:5147–5160. [PubMed: 17515611]

Tan J, Yang X, Zhuang L, Jiang X, Chen W, Lee PL, et al. Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. Genes Dev. 2007; 21:1050–1063. [PubMed: 17437993]

Tanemura A, Terando AM, Sim MS, van Hoesel AQ, de Maat MF, Morton DL, et al. CpG island methylator phenotype predicts progression of malignant melanoma. Clin Cancer Res. 2009; 15:1801–1807. [PubMed: 19223509]

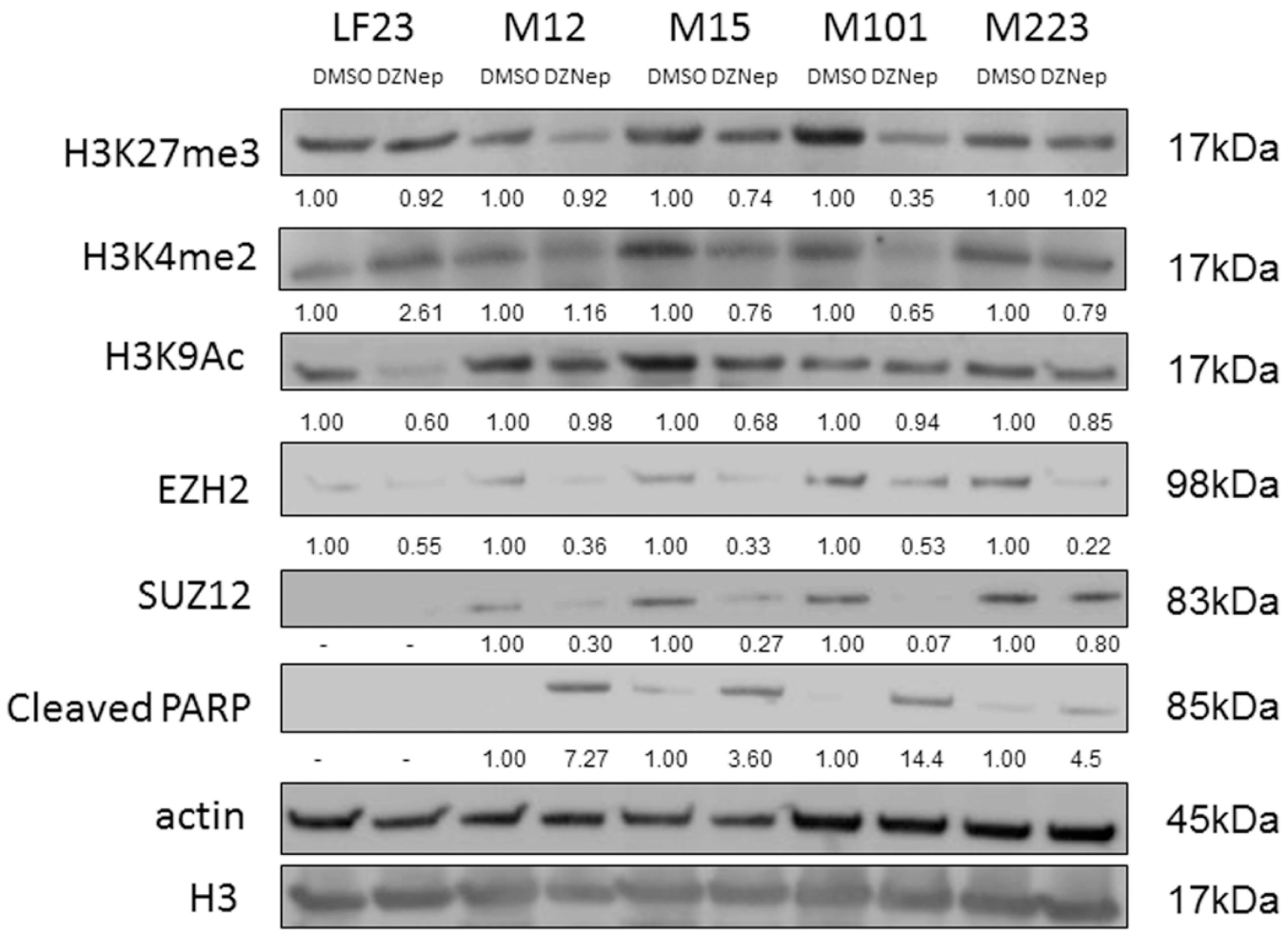

Figure 1. The effects of DZNep in five melanoma cell lines

Five melanoma lines were treated with DMSO(control) or 5μM DZNep for 72h. Whole-cell lysates were examined by Western blot. Treatment of M12, M15, M101, and M223 cells with 5μM DZNep for 72h led to the repression of H3K27me3 and PRC2 components, EZH2 and SUZ12. In DZNep-treated cells, cleaved PARP was detected. β-Actin protein was used as a gel loading control.