UVA and UVB Induce Different Sets of Long Noncoding RNAs


TO THE EDITOR

Although 76% of the human genome is transcribed, only 2.94% encodes messenger RNA (Pennisi, 2012; The ENCODE Project Consortium, 2012). The vast majority of transcribed RNAs are noncoding RNAs (Cech and Steitz, 2014). The total number of long noncoding RNAs (lncRNAs), which are more than 200 nucleotides long, is estimated to be close to 60,000 (Iyer et al., 2015). According to the Long Non-Coding RNA Database (http://www.lncrnadb.org), the functions of only less than 0.5% of lncRNAs are known. Of those lncRNAs, many regulate the expression of protein-coding genes.

UVR has many effects on skin, including pigmentation, immune modulation, vitamin D production, photocarcinogenesis, and photoaging. On a molecular level, UVR damages cellular molecules, for example, DNA. Cells are equipped to counteract the damaging effects of UVR and to repair cellular damage. After UVR exposure, the expression of many protein-coding genes is altered to orchestrate these damage responses. Long-wave (UVA, 315–400 nm) and short-wave (UVB, 280–315 nm) UVR differ in their photophysical and photochemical properties and also entail different cellular responses (Halliday and Rana, 2008; Rünger et al., 2012; Syed et al., 2012).

Some of the UVR-induced changes in gene expression are driven by central coordinators of cellular damage responses, for example, the kinases ATM and ATR and the transcription factors p53, NF-kB, and AP-1. Damage sensors have been postulated to initiate cellular damage responses, but it is only incompletely understood how the activation of these sensors translates into changes in the expression of thousands of genes.

lncRNAs have been shown to play important roles in development, aging, wound healing, and in a number of diseases (Liu et al., 2014; Pefanis et al., 2015; Wu et al., 2015; Zhou and Xu, 2015). However, there are only very few reports describing changes in the expression of lncRNAs in response to cellular insult, and only very few single lncRNAs have been investigated in response to UVR in particular (Hall et al., 2015; Szegedi et al., 2012).

Given that UVR has profound effects on the gene expression profiles of exposed cells and the increasingly recognized role of lncRNAs to regulate gene expression, we hypothesized that lncRNAs mediate many of the changes in gene expression and the subsequent functional responses to UVR. We therefore performed a genome-wide analysis of lncRNAs using a microarray specifically designed for the detection of lncRNAs after exposure of skin cells to UVR (Supplementary Materials and Methods online). Doses were chosen based on dose-finding experiments to ensure similar toxicity of the different experimental conditions and to avoid excessive toxicity that may not be specific for cellular responses to UVR (Supplementary Figure S1 online). Because cellular responses to UVA and UVB are profoundly different, we hypothesized that the lncRNA response to UVA and UVB would also be different and therefore investigated the lncRNA response separately for UVA and UVB. To mimic the effects of long-term UVR exposure, we also included repeated exposures, in addition to single doses of UVA or UVB.

A total of 660 lncRNAs were up- or downregulated by UVA (Figure 1). The entire microarray data set can be accessed at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE89005. Heat maps demonstrate good reproducibility for most genes in the triplicate unirradiated control samples and the duplicate irradiated samples (Figure 1a). These heat maps cannot be used for pathway analysis, because the functions of most lncRNAs are unknown. Venn diagrams show that the majority of lncRNA genes are only affected either by UVA or UVB, and that only few lncRNA genes are up- or downregulated by both (Figure 1b). This indicates that cells respond to UVA and UVB in a profoundly different way with alteration of mostly different sets of lncRNAs.

The high number of lncRNA genes up- or downregulated by UVA or UVB precluded quantitative real-time polymerase chain reaction confirmation for all genes. We therefore identified the most promising 21 lncRNA genes using the following criteria: 1. up- or downregulated more than twofold; 2. P value < 0.05; 3. top three up- or downregulated lncRNAs for each condition; 4. top two up- or downregulated lncRNAs that are also up- or downregulated with other conditions; 5. similar results with different probes for the same gene (there is more than one probe for approximately 97% of detected lncRNA genes on the microarray); 6. different sham controls (single and repeated irradiation) show similar results.

Of the 21 lncRNAs, quantitative real-time polymerase chain reaction confirmed the up- or downregulation of only 4 lncRNA genes: the lncRNAs GS1-600G8.5 and RPT1-631K18.2, which were induced by a single exposure to UVA; LOC389641, which was induced by a single exposure to UVB; and IMMP2L-IT1, which was repressed by repeated exposures to UVB (Figure 2a–d). These changes in lncRNA expression were wavelength-dependent.
specific: The lncRNAs affected by UVA were not altered by UVB, and vice versa. Fluorescence in situ hybridization confirmed the upregulation of LOC389641 by UVB in cultured skin fibroblasts (Figure 2e), as well as in keratinocytes and full skin (Supplementary Figure S2 online). Probes for the detection of the other lncRNAs by fluorescence in situ hybridization failed to detect expression in sham- or UV-irradiated cells.

This is, to our knowledge, the first genome-wide analysis of lncRNA expression changes in response to UVA and UVB. It points to an extensive system of lncRNA-dependent gene regulation and suggests that lncRNAs are critical mediators of damage responses to UVR.

Given the large difference in how cells respond to UVA and UVB, it is not surprising that the lncRNA gene expression profiles after exposure to UVA and UVB are also very different. After UVB, the expression of thousands of coding genes is altered (Koch-Paiz et al., 2004) and this is mirrored by our result of expression changes in several thousands of lncRNA genes. Likewise, expression changes in hundreds of coding genes with UVA (Koch-Paiz et al., 2004) are mirrored by expression changes in hundreds of lncRNA genes. In addition, our result that there is only very little overlap...
between UVA- and UVB-induced IncRNA genes provides further evidence that cellular responses to UVA and UVB are profoundly distinct.

CONFLICT OF INTEREST
This work was funded by a research training grant from POLA Chemical Company, Inc. to TMR. KY is an employee of POLA Chemical Company, Inc.

Kazuyuki Yo1,2 and Thomas M. Rünger1,*
1Department of Dermatology, Boston University School of Medicine, Boston, Massachusetts, USA; and 2Dermatological R & D, Skin Research Department, POLA Chemical Industries, Inc., Totsuka-ku, Yokohama, Japan
*Corresponding author e-mail: truenger@bu.edu

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.10.041.

REFERENCES
Liu JY, Yao J, Li XM, Song YC, Wang XQ, Li YJ, et al. Pathogenic role of IncRNA-MALAT1 in...

RNA-Binding Protein IGF2BP1 in Cutaneous Squamous Cell Carcinoma

TO THE EDITOR

Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer, accounting for 20% of non-melanoma skin cancer. Although most small cSCCs are readily curable by surgical approaches, surgery is not effective for patients with advanced and metastasized cSCC. A prospective study showed that the rate of nodal metastasis and disease-specific death for cSCC are 4% and 1.5%, respectively (Brantsch et al., 2008). Thus, identification of additional therapeutic targets remains imperative for inoperable SCC.

IGF2BP1 is a multifunctional RNA-binding protein that has been shown to affect multiple prosurvival, anti-apoptotic, and drug resistance pathways (Craig and Spiegelman, 2012; Elcheva et al., 2008; Noubissi et al., 2010). IGF2BP1 has an oncofetal pattern of expression and although it is important for normal embryonic development, in normal tissues it is expressed at very low levels, and its down-regulation in vitro and in vivo has no significant physiological effect. Conversely, we have found that ectopic expression of IGF2BP1 in normal keratinocytes leads to increased proliferation and inhibition of apoptosis (Noubissi et al., 2014). IGF2BP1 has also been shown to drive tumorigenesis in vivo (Tessier et al., 2004). IGF2BP1 was shown to regulate several proto-oncogenes and oncogenic signaling pathways that are activated in SCC (Bhatia and Spiegelman, 2005; Noubissi et al., 2006; Pierceall et al., 1991). In this report we analyzed the role of IGF2BP1 in the pathogenesis of cSCC.

To assess the levels of IGF2BP1 in human cSCC, we isolated RNA from fresh frozen cSCC tissues and matching control skin from 68 patients who presented for Mohs micrographic surgery. All human studies have been approved by our institutional review board, and patients have given their written informed consent. Quantitative reverse transcriptase–PCR showed that IGF2BP1 mRNA was significantly overexpressed in SCCs compared with their matching controls (Figure 1a, and see Supplementary Figure S1 online). We also measured mRNA levels of two IGF2BP1-binding targets, β-TrCP1 and c-Myc, which play roles in cell cycle progression, growth and apoptosis. Both were overexpressed in most patients along with IGF2BP1 overexpression, and statistical analysis confirmed a positive correlation between expression levels of IGF2BP1 and both β-TrCP1 and c-Myc (Figure 1c and d). Immunoblot analysis confirmed that IGF2BP1 protein was overexpressed in SCC samples compared with matching controls (Figure 1b).

To further test the association of IGF2BP1 and clinical features, the following patient information was collected: age, sex, lesion size at time of biopsy, lesion size before Mohs surgery, defect size after Mohs surgery, keratoacanthoma type, cell differentiation (well, moderate, and poor), transplant versus non-transplant recipient, and stage (T0, T1, T2a, T2b, and T3) based on Brigham and Women’s Hospital staging system (Jambusaria-Pahlajani et al., 2013). Some tumors may be understaged because our study does not allow a complete assessment of tumor depth histologically. We also attempted to collect clinical outcome data (local recurrence, regional metastasis, distant metastasis, or death) with a follow-up duration of 1–4 years; only four patients had adverse outcomes related to SCC. We performed univariate regression models of log2 fold change on these various parameters. We found that poorly differentiated cells expressed higher levels of IGF2BP1 mRNA than moderately and well-differentiated cells (P = 0.008, F test) (see Supplementary Figure S2a and Supplementary Table S1 online). Also, models showed that increase in tumor staging is associated with increase in IGF2BP1 mRNA expression level (P = 0.017, F test) (see Supplementary Figure S2b and c and Supplementary Table S1). Statistically significant association was not found between IGF2BP1 expression and the other listed parameters, including clinical outcomes. These data collectively show that IGF2BP1 and its targets are overexpressed in human cSCC. The levels of IGF2BP1 expression inversely correlate with cell differentiation and

Abbreviations: cSCC, cutaneous squamous cell carcinoma; SNP, single nucleotide polymorphism
Accepted manuscript published online 14 November 2016; corrected proof published online 14 November 2016
© 2016 The Authors. Published by Elsevier, Inc. on behalf of the Society for Investigative Dermatology.