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UVA-Irradiation Induces Melanoma Invasion via the Enhanced Warburg Effect

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Melanoma is a malignant tumor in which UVA (320–400 nm) radiation is considered to be an important risk factor. But the role of UVA in melanoma progression toward an invasive phenotype is still not adequately investigated. For most proliferating tumor cells the preference of aerobic glycolysis has been described as the Warburg effect. Here we investigate the effect of UVA irradiation on changes in the Warburg effect and tumor progression toward invasive potential. On UVA irradiation, melanoma cell lines from initial tumors show an induction of the Warburg effect with increased glucose consumption and lactate production, which is at least partially mediated by reactive oxygen species. Associated with UVA treatment and enhanced lactic acid production, tumor-relevant proteases and in situ invasion is upregulated. Simultaneously, UVA increases intracellular concentrations of progression marker transketolase and activated protein kinase Akt, both involved in metabolic changes that increase with proliferation. Using invasion assays we show that lactic acid, resulting from the UVA enhanced and partially reactive oxygen species-mediated Warburg effect, increases the invasive potential of all melanoma cell lines investigated. Therefore, we demonstrate in melanoma cells that production of lactic acid, induced by UVA irradiation, increases invasiveness of melanoma cells via expression of tumor-relevant proteases.

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INTRODUCTION

Melanoma is a malignant skin tumor characterized by high morbidity and mortality (Howe et al., 2001; Jemal et al., 2010; Jhappan et al., 2003; Leiter et al., 2014). Melanomas develop from initial tumor cells via radial and vertical growth, ultimately leading to metastasis, and epidemiological evidence indicates that UV radiation is involved in the generation of melanoma (Bald et al., 2014; Fears et al., 2002; Tucker and Goldstein, 2003). Recently it was shown that UVB irradiation enhances perivascular invasion of melanoma cells (Bald et al., 2014). Although this UVB irradiation correlates with sunburn reactions in the skin, solar UV radiation with physiological relevance consists, to the largest extent, of UVA radiation (320–400 nm) (Baczynska et al., 2013; Parisi and Wong, 2000; Turnbull and Parisi, 2003). The effect of UVA irradiation is partially mediated by reactive oxygen species (ROS), finally leading to intracellular oxidative damage (Beissert and Loser, 2008; Kappes et al., 2006). This is of particular importance because—as opposed to UVB—UVA reaches basal layers of the epidermis containing melanocytes as well as the dermis at pathophysiologically relevant doses. Despite its physiological relevance, the contribution of UVA to solar-induced melanoma is still discussed controversially as studies with a Xiphophorus hybrid fish model could not detect UVA-induced melanomas (Mitchell et al., 2010). In addition to this, epidemiological data report melanoma on usually sun-protected body sites (Levell et al., 2009), but there are also epidemiological data that show a clear correlation between sunburn and melanoma in sun-sensitive patients (Newton-Bishop et al., 2011). Furthermore, a single neonatal UVA irradiation, mimicking sunburn reactions in childhood, in a transgenic mouse model on an albino inbred background did not significantly induce melanomas (De Fabo et al., 2004; Noonan et al., 2001; Zaidi et al., 2011). But interestingly in pigmented transgenic mice, a single neonatal dose of UVA irradiation (Noonan et al., 2012) induces oxidative DNA damage in melanocytes and is sufficient to induce melanotic tumors. Although these studies strongly support the role of a single neonatal high dose of UVA during melanoma pathogenesis, they do not model other modes of UV exposure, such as the repeated exposure of humans to low doses of solar UV irradiation in everyday life. In addition, it is still not clear which role exposure to UVA irradiation plays during early melanoma progression. Two important features during progression of initial melanoma are (i) invasion, needing specific proteases, and (ii) proliferation, needing a specific metabolic setting.

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Abbreviations: ANOVA, analysis of variance; 2-DG, 2-deoxy-D-glucose; IM, initial melanoma; MM, metastasizing melanoma; MMP, matrix metalloproteinase; ROS, reactive oxygen species; SD, standard deviation; TKTL-1, transketolase 1; TIMP1, tissue inhibitor of metalloproteinase; uPA, urokinase-type plasminogen activator

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Proliferating cells, including tumor cells, turn to glycolysis for energy production with subsequent lactate fermentation even in the presence of oxygen, a metabolic characteristic described by Warburg (1927), thus called the Warburg effect. Increased glycolysis is associated with the activation of Akt (Elstrom et al., 2004), a protein kinase also involved in anti-apoptotic signaling (Majewski et al., 2004). Interestingly, Akt phosphorylation at serine 473 is present in the majority of immunohistologically investigated melanomas (Dhawan et al., 2002). Another tumor-relevant metabolic change is the activation of the pentose phosphate pathway that provides tumor cells with components for nucleotide synthesis. A key enzyme of this pathway is transketolase like 1 (TKTL1), which is used as a tumor marker for highly proliferative cancers (Diaz-Moralli et al., 2011; Langbein et al., 2006). An important step in melanoma progression is invasion of adjacent tissue, facilitated by proteases such as matrix metalloproteinases (MMPs) and urokinase-type plasminogen activator (uPA). Particularly, it was shown that MMP9 and uPA are activated during invasion of melanoma cells (Bianchini et al., 2006; Tang et al., 2013).

To investigate the role of UVA irradiation for melanoma progression and invasion via the Warburg effect, we exposed cell lines of initial melanoma with chronic sublethal doses of UVA. Here we show a UVA-dependent increase in glucose uptake and lactate production, and a pH decrease. This lactate production subsequently led to increased expression of MMPs and uPA, resulting in increased invasiveness of UVA-treated melanoma cells. We provide a functional link between the UVA-induced Warburg effect with enhanced lactic acid production and enhanced expression of MMP and uPA, which finally promotes enhanced invasion.

RESULTS

The UVA-induced Warburg effect mediated by ROS

The Warburg effect is characterized by a preference for glycolysis (enhanced glucose consumption) and subsequent lactic acid fermentation. Melanoma cells from initial melanomas (IM) and metastasizing melanomas (MM) (Supplementary Table S1 and Supplementary Figure S1 online) were irradiated with UVA (6 J/cm^2) three times daily for 4 consecutive days. Glucose consumption and lactate production of IM and MM cells were measured in the absence or presence of UVA exposure. On UVA irradiation all melanoma cell lines showed increased (Student's *t*-test; $P < 0.01$) glucose consumption (Figure 1a and c) and lactate production (Figure 1b and d) (mean value and standard deviation [SD] of at least four independent experiments and Student's *t*-test shown; asterisks represent $P < 0.01$ and $P < 0.05$). This effect was also observed for different doses of UVA irradiation in IM and MM cells, partially showing a significant dose-dependent upregulation of the Warburg effect (analysis of variance [ANOVA], post-test for linear trend) (Supplementary Figure S2a and b online). Despite different genetic backgrounds, every IM and MM investigated tended, when compared with unirradiated control, to enhanced UVA-induced glucose consumption (Figure 1e) and lactate production (Figure 1f) (mean value and SD of at least three independent experiments shown). As a consequence of the increased release of lactic acid in the medium, the pH in the medium decreased compared with unirradiated control (Figure 1g and h) (mean value and SD of at least three independent experiments shown and Student's *t*-test shown; asterisks represent $P < 0.01$).

Because exposure to UVA irradiation generates ROS (Meewes et al., 2001), we tested whether the UVA-induced Warburg effect in melanoma cells is ROS mediated by coincubation with ROS quencher Trolox. The addition of 20 μM Trolox significantly decreases the UVA-induced glucose consumption and lactate production (Figure 1i and j) (mean value and SD of at least three independent experiments and two-way ANOVA, Bonferroni multiple comparisons post-test shown; asterisk represents $P < 0.05$).

The inhibitor of glycolysis 2-deoxy-D-glucose (2DG) reversely blocks activity of glucose kinase, a key enzyme of the glycolytic pathway. In consequence, we found that 5.5 mM of 2DG significantly diminished (Student's *t*-test; $P < 0.05$) UVA-induced glucose consumption and increased lactate production (Figure 1k and l) (glucose consumption and lactate production presented as the mean value with SD of triplicates as representative of at least four independent experiments).

To recapitulate the situation in human skin in vivo more closely, skin reconstructs (Backvall et al., 2002) with different melanoma cells (IM and MM) treated either with or without repetitive UVA doses showed more lactate production on UVA irradiation compared with unirradiated control (Figure 1m–o) (mean value and SD of at least three independent experiments and Student's *t*-test shown; asterisks represent $P < 0.01$ and $P < 0.05$).

UVA irradiation enhances transketolase activity

Transketolase isoform TKTL1 is a key enzyme of the pentose phosphate pathway and part of the metabolic network supporting growth of tumor cells (Resendis-Antonio et al., 2010). To investigate the effect of UVA irradiation on TKTL1 expression, melanoma cells (IM and MM) were UVA-irradiated (as described above) and expression of TKTL1 was detected by immunocytochemical staining. All melanoma cell lines (IM and MM) show enhanced TKTL1 expression on UVA irradiation (Supplementary Figure S2d and e). Simultaneously, the same UVA treatment increased total transketolase activity in melanoma cells (IM and MM) in a colorimetric assay (Smith et al., 2006). Increased transketolase activity on UVA treatment (Figure 2a) was visible in every investigated IM and MM, independent from their different genetic background (mean value and SD of three independent experiments). This UVA-enhanced transketolase activity in IM melanoma cells was dependent on UVA-induced ROS, as the addition of Trolox attenuated UVA-induced transketolase activity (Figure 2b and c) (mean value and SD of at least three independent experiments and two-way ANOVA, Bonferroni multiple comparisons post-test shown; asterisk represents $P < 0.05$). Furthermore, UVA-enhanced transketolase activity showed partially a dose-dependent upregulation of transketolase activity (Supplementary Figure S2c).

Skin reconstructs with different melanoma cells (IM and MM) treated either with or without repetitive UVA doses also showed elevated transketolase activity on UVA irradiation