Molecular Basis of Tobacco Smoke-Induced Premature Skin Aging

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Although it is now widely recognized that tobacco smoke has negative effects on the skin, the molecular mechanisms underlying its skin-aging effects remain uncertain. Epidemiological studies indicate that tobacco smoking is a strong independent predictor of facial wrinkle formation and other aspects of premature skin aging. Recent *in vivo* studies in humans and mice provided the first direct evidence that tobacco smoke causes premature skin aging, and they have begun to reveal the molecular changes in the skin that occur in response to it. Water-soluble tobacco smoke extract, which predominantly produces oxidative stress when applied topically to cultured skin fibroblasts, impairs collagen biosynthesis. Matrix metalloproteinases, which degrade collagen, are induced dose-dependently by tobacco smoke extract as well as by other constituents that trigger the aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor that mediates the toxicity of several environmental contaminants, including photoproducts in the body generated by UVB radiation. Tobacco smoke also contains many non-water-soluble constituents that activate the AhR pathway. Our most recent studies using hexane-soluble tobacco extract indicate that activation of the AhR pathway may play a role in the premature skin-aging effects of tobacco smoke exposure.

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INTRODUCTION

Tobacco smoking has specific damaging effects on the skin, which can result in poor wound healing, squamous cell carcinoma, melanoma, oral cancer, acne, psoriasis, eczema, hair loss, and premature skin aging (Freiman *et al.*, 2004). Epidemiological studies implicate tobacco smoking as an important factor in premature skin aging (Kadunce *et al.*, 1991; Ernster *et al.*, 1995; Morita, 2007). Tobacco smoking induces structural and compositional changes in the epidermis and dermis similar to those resulting from chronic UV radiation exposure, and it is an important environmental factor in premature skin aging (Grether-Beck *et al.*, 1997; Fisher *et al.*, 1999). A smoker's face is characterized by gray skin (smoker's melanosis) and deep wrinkles (smoker's wrinkle). Here, we describe molecular mechanisms underlying tobacco smoke-induced premature skin aging (Figure 1).

IN VIVO EVIDENCE FOR THE SKIN-AGING EFFECTS OF TOBACCO SMOKE

Wrinkle formation is a typical clinical feature associated with tobacco smoking (Daniell, 1971). In a cross-sectional study investigating the association between facial wrinkle formation and tobacco smoking in Japan (Yin *et al.*, 2001), subjects completed a questionnaire to assess their sun exposure, pack-years of smoking history, and potential confounding variables. Facial wrinkles were quantified using the Daniell score, and stepwise regression analysis of the score produced the following formula: Daniell score $= -1.24 + 0.05 \times$ age $+ 0.015 \times$ pack-year $+ 0.158 \times$ sun exposure. Logistic regression analysis of the data indicated that age (odds ratio = 7.5, 95% confidence interval = 1.87–30.16), pack-year (odds ratio = 5.8, 95% confidence interval = 1.72–19.87), and sun exposure (odds ratio = 2.65, 95% confidence interval = 1.0–7.0) independently contribute to facial wrinkle formation (Morita, 2007). Further, tobacco smoking, and not UV exposure, is a strong predictor of skin aging (Leung and Harvey, 2002).

Significantly higher levels of matrix metalloproteinase (MMP-1) mRNA are present in the buttock dermal connective tissue of smokers compared with non-smokers (Lahmann *et al.*, 2001). MMP-1 induces collagen and elastic fiber degradation. We recently applied water-soluble tobacco smoke extract to male hairless mice topically or intracutaneously to the back, or intraperitoneally three times a week for 6 months. Tobacco smoke extract applied topically or intracutaneously induced a loss of collagen staining and a concomitant increase in the ground substance in the upper dermis, indicative of collagen damage. Intraperitoneal injection had no effect. Although a relationship between smoking

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Abbreviations: AhR, aryl hydrocarbon receptor; Arnt, AhR nuclear translocator; MMP, matrix metalloproteinase; PAH, polycyclic aromatic hydrocarbon; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin

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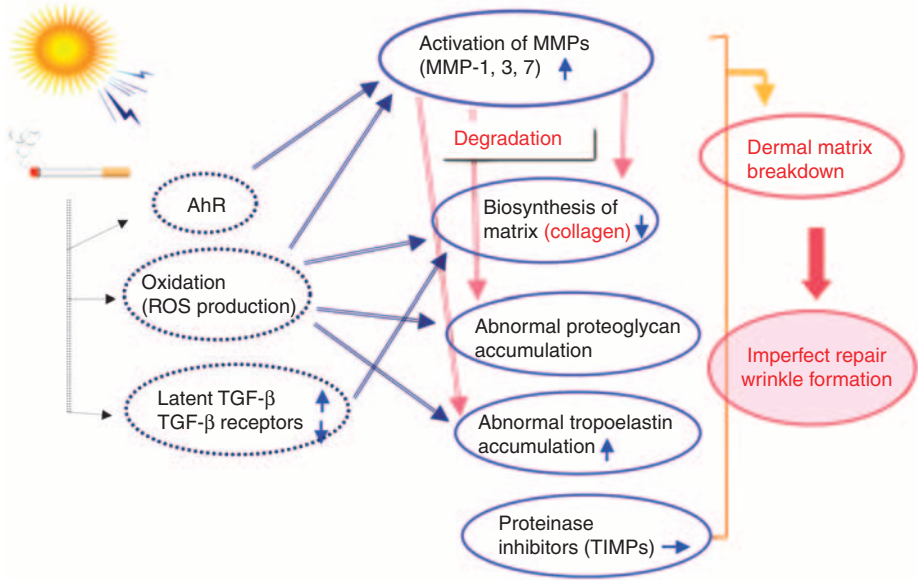

Figure 1. Molecular mechanisms of tobacco smoke-induced premature skin aging.

tobacco and skin wrinkling had been shown previously, these findings were the first direct evidence from an *in vivo* study that tobacco smoke induces premature skin aging (Tanaka *et al.*, 2007).

MOLECULAR EVIDENCE THAT TOBACCO SMOKE IMPAIRS COLLAGEN METABOLISM

We next examined whether tobacco smoke extract affects collagen directly by studying its effect on collagen metabolism in human skin fibroblasts. Tobacco smoke extracts impaired collagen biosynthesis significantly in cultured skin fibroblasts (Yin *et al.*, 2000). In addition, production of the collagen precursors, procollagen types I and III, was decreased significantly in supernatants of cultured fibroblasts treated with tobacco smoke extracts, and MMP-1 and MMP-3 were induced in a dose-dependent manner (Yin *et al.*, 2000). The expression of tissue inhibitors of metalloproteinase remained unchanged, however, indicating that tobacco smoke extract altered the ratio between these compounds in favor of MMPs (Yin *et al.*, 2000). Therefore, tobacco smoke not only impairs collagen biosynthesis, but also causes collagen degradation by inducing MMPs.

Tobacco smoke extract also induces the non-functional latent form of transforming growth factor-β in supernatants of cultured skin fibroblasts (Yin *et al.*, 2003). Cellular responsiveness to transforming growth factor-β1 is blocked by this non-functional form, and the downregulation of the transforming growth factor-β1 receptor results in decreased synthesis of extracellular matrix proteins.

TOBACCO SMOKE AND THE ARYL HYDROCARBON RECEPTOR

In the above studies, we used a water-soluble tobacco smoke extract that induces oxidative stress predominantly in cultured fibroblasts (Yin *et al.*, 2000). Tobacco smoke is comprised of at least 3,800 constituents, including numerous water-insoluble polycyclic aromatic hydrocarbons (PAHs) that trigger the aryl hydrocarbon receptor (AhR) signaling pathway.

Benzo[a]pyrene, a specific component within the class of PAHs, was the first chemical carcinogen discovered in tobacco smoke (Proctor, 2006). Tobacco smoke is a significant source of PAH exposure, and PAHs are implicated to be the principle carcinogenic agents in tobacco-related lung cancer (Proctor, 2006).

The PAH 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has several pathological effects in humans through the activation of the AhR pathway. The primary mechanism underlying the changes in gene expression induced by TCDD is activation of the transcription pathway comprising AhR and the AhR nuclear translocator (Arnt). AhR and Arnt are transcription factors involved in the regulation of development, hypoxia signaling, and circadian rhythms, and belong to a family of proteins that reside in the cytoplasm in an inactive complex with accessory proteins (Carver *et al.*, 1998; Kazlauskas *et al.*, 2000). Once bound to TCDD, AhR dissociates from some of the proteins in the inactive complex and translocates to the nucleus where it dimerizes with Arnt (Reyes *et al.*, 1992). The AhR/Arnt heterodimer activates the transcription of xenobiotic-metabolizing genes (Fujii-Kuriyama *et al.*, 1992; Watson and Hankinson, 1992), some of which encode proteins involved in growth control, cytokines, nuclear transcription, and regulators of extracellular matrix proteolysis (Sutter *et al.*, 1991; Yin *et al.*, 1994). Therefore, the AhR pathway may be involved in the effects of tobacco smoke on skin.

POSSIBLE LINK BETWEEN AhR AND PREMATURE SKIN AGING

The AhR pathway is activated by TCDD and all-trans retinoic acid, both of which increase MMP-1 expression in normal human keratinocytes (Murphy *et al.*, 2004), suggesting that MMP induction is a common mechanism underlying TCDD-induced pathologies. MMP is regulated predominantly at the level of gene

transcription and activity (Vincenti *et al.*, 1996), and consensus activator protein-1 is involved in MMP-1 transcriptional activation. TCDD-induced MMP-1 expression is mediated through two activator protein-1 elements in the proximal promoter of the MMP-1 gene (Auble and Brinckerhoff, 1991; Benbow *et al.*, 1999). TCDD-induced expression of MMP-1 requires both activator protein-1 elements (Murphy *et al.*, 2004).

Human melanocytes and some of the more metastatic melanoma cell lines are activated by TCDD, express both AhR and Arnt, and increase MMP expression and activity (Benbow *et al.*, 1999). TCDD-induced MMP-1 expression of metastatic melanoma cells *in vitro*, however, does not require activator protein-1 elements, but is mediated through sequences in the distal promoter region.

Another environmental factor, UVB radiation, generates the endogenous AhR ligand 6-formylindolo[3,2-*b*]carbazole from tryptophan (Fritsche *et al.*, 2007), which may be a photoproduct that initiates signaling events transferred to the nucleus and cell membrane through the activation of cytoplasmic AhR. UVB irradiation of HaCaT keratinocytes induces cytochrome P1A1 (CYP1A1) mRNA expression, which is inhibited through the depletion of tryptophan or by directly interfering with AhR signaling. These studies indicate that AhR is critically involved in UVB-induced CYP1A1 mRNA induction (Fritsche *et al.*, 2007).

To analyze the involvement of AhR in tobacco smoke-induced skin aging, we exposed primary human fibroblasts to hexane-soluble tobacco smoke extract. This extract increased MMP-1 mRNA induction significantly in cultured human fibroblasts, in association with a significant upregulation of cytochrome P1B1 (CYP1B1) expression. AhR knockdown abolished the increased transcription of the AhR-dependent gene, CYP1A1/CYP1B1, induced by the extract. CYP1B1 and MMP-1 induction was abolished by the AhR pathway inhibitors 3-methoxy-4-nitroflavone and α-naphthoflavone (Ono *et al.*, 2008).

These studies suggest that hexane-soluble tobacco smoke extract induces MMP-1 expression in human skin fibroblasts through the activation of the AhR pathway. Thus, the AhR pathway may be pathogenetically involved in extrinsic skin aging.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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