Expression of CCN family of genes in human skin in vivo and alterations by solar-simulated ultraviolet irradiation

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Abstract The CCN family of proteins is involved in diverse biological functions such as cell growth, adhesion, migration, angiogenesis, and regulation of extracellular matrix. We have investigated expression of CCN family genes and alterations induced by solar-simulated ultraviolet irradiation in human skin in vivo. Transcripts of all six CCN genes were expressed in human skin in vivo. CCN5 was most abundantly expressed followed by CCN2>CCN3>CCN1>CCN4>CCN6. Solar-simulated ultraviolet irradiation increased mRNA expression of CCN1 and CCN2. In contrast, mRNA levels of CCN3, CCN4, CCN5, and CCN6, were reduced. Knowledge gained from this study provides the foundation to explore the functional roles of CCN gene products in cutaneous biology and responses to solar ultraviolet irradiation.

Keywords CCN · UV · Skin

Introduction

Human skin, the largest organ of the body, serves as a protective barrier to environmental damage. Skin is directly exposed to ultraviolet (UV) irradiation from the sun. Solar UV irradiation is a potent environmental factor that can deleteriously affect the structure and function of human skin. Acute exposure of human skin to UV irradiation causes sunburn, altered pigmentation, inflammation, immune suppression, and dermal connective tissue damage (Gilchrest and Yaar 1992; Kripke 1984; Matsumura and Ananthaswamy 2002; Pathak 1986). Chronic exposure to UV irradiation over many years disrupts normal skin and ultimately causes premature skin aging (photoaging) (Fisher et al. 1996; Fisher et al. 1997; Yaar and Gilchrest 2007) and cancer (Gloster and Brodland 1996; Green and Hedinger 2008; Katiyar et al. 2000; Miller and Mihm 2006).

CCN family proteins are secreted, matricellular signaling molecules. CCN proteins are capable of mediating diverse biological functions including cell growth, adhesion, migration, angiogenesis, and regulation of extracellular matrix (Chen et al. 2001; Kireeva et al. 1996; Perbal et al. 2003). CCN family of proteins currently consists of six highly conserved genes; CCN1/CYR61, CCN2/CTGF, CCN3/NOV, CCN4/WISP-1, CCN5/WISP-2, and CCN6/WISP-3 (Brigstock 1999; Brigstock et al. 2003). Altered expression of CCN genes is associated with numerous pathological states including fibrotic disorders and tumorigenesis (Brigstock et al. 2003; Leask and Abraham 2003; Perbal 2004; Planque and Perbal 2003). CCN proteins were discovered more than two decades ago (Bradham et al. 1991; Joliot et al. 1992; O’Brien et al. 1990), however, expression of CCN genes and their regulation by UV irradiation have not been studied in human skin in vivo. Here we quantified expression of the six CCN family genes, and further explored the impact of acute UV-irradiated on CCN gene expression in human skin in vivo.

Methods

UV irradiation and procurement of human tissue samples

Human skin punch biopsies were obtained from healthy,
adult human volunteers, as previously described (Fisher et al. 1998; Fisher et al. 1997; Fisher and Voorhees 1998; Fisher et al. 1997). For UV irradiation, sun-protected buttock skin was irradiated with 2 MED (minimum erythema dose) solar-simulated UV (SPEC 450 W xenon arc solar simulator) (Quan et al. 2004). Skin samples (4 mm diameter) were obtained at the indicated time points after UV irradiation exposure. All procedures involving human subjects were approved by the University of Michigan Institutional Review Board, and all subjects provided written informed consent.

**RNA isolation and quantitative real-time reverse transcription polymerase chain reaction (RT-PCR)** Total RNA was isolated from human skin samples using a commercial kit (RNeasy Mini Kit, Qiagen, Chatsworth CA) according to the manufacturer’s protocol. Total RNA was quantified using RiboGreen™ RNA Quantitation Reagent and Kit (Molecular Probes, Eugene, OR), and was reverse transcribed using Taqman Reverse Transcription kit (Applied Biosystems, Foster City, CA). Quantitative real-time PCR was performed using Taqman Universal PCR Master Mix Reagents and ABI Prism 7700 Sequence Detector (Applied Biosystems). PCR primers and probes for 36B4 (internal control) was published as previously described (Quan et al. 2002a; Quan et al. 2006).

Multiplex PCRs were performed with each primer and probe set and 36B4 (internal control). Expression of each gene and 36B4 mRNA curves. cDNA plasmid standards for each individual genes were cloned using a pcRII® -TOPO® vector (Invitrogen, Carlsbad, CA). PCR cloning primers for CCN family genes are described in Table 2.

**Statistical analysis**

Comparisons between groups were determined with the Student’s t-test. All p values are two-tailed, and considered significant when \( p < 0.05 \).

**Results**

**Expression of CCN genes in normal human skin in vivo** To explore expression of CCN genes in human skin in vivo, total RNA was prepared from full-thickness punch biopsies

| Table 1 | Primer and probe nucleotide sequence for quantitative real-time RT-PCR analysis |
|---------|-------------------------------------------------------------------------------|
| Gene identity | Primer sequence (5′–3′) | Probe sequence (5′–3′) | Size (bp) | Accession # |
| CCN1/CYR61 | Forward: TCAAAGACCTGTTGGAACCTGTTAC | CAATGACAACCTGAGTTGACCT | 85 | AF307890 |
| | Reverse: CAGAATACCGTTCTCTTTC | | | |
| CCN2/CTGF | Forward: GGTGGCCAGACACCTCCT | TGATTAGGCAACTGCTTGCTCA | 70 | NM_001901 |
| | Reverse: GGAACAGGCTCACCACCTC | | | |
| CCN3/NOV | Forward: CGCCCTCAGCTGTTGATG | ACTGGTTCAGCTCTACCTGAC | 71 | AY082381 |
| | Reverse: CTCAAGCTCACCACCTC | | | |
| CCN4/WISP1 | Forward: AGAGGCCAGCCACCTGTTAC | CGGGCTGATCAGCAGTAC | 75 | AF100779 |
| | Reverse: CAACTCCAGCAGTCTGTTGGA | | | |
| CCN5/WISP2 | Forward: ATGAGAGGCACAACCCAGAC | CACCTCTCGCTCTTCTCCCTTC | 72 | NM_003881 |
| | Reverse: CTGGTTCAGCACCTTTCGAGA | | | |
| CCN6/WISP3 | Forward: CCTATCATACTGCTCTTGTTCA | CCAAACCTTTGTCAAGCCTGCT | 70 | AF100781 |
| | Reverse: CAAGTGGCCACCCCTCACCA | | | |

| Table 2 | Primer nucleotide sequence for cloning CCN cDNA plasmid for quantitative real-time RT-PCR analysis |
|---------|----------------------------------------------------------------------------------|
| Gene identity | Forward primer sequence (5′–3′) | Reverse primer sequence (5′–3′) | Size (bp) |
| CCN1/CYR61 | ACGGCTGCGGGCAGCTGTTAGTGC | AAGGGCGCACTCAGGGTGTCAT | 638 |
| CCN2/CTGF | ACGAGCCCAAGAGCAGCCACCTG | ACAGTTGTAATGGCCAGCAC | 488 |
| CCN3/NOV | TGACCGGCGGTAGGAGGAGATAC | TGGTTTAGGTGAGGCTTGGAGTAC | 527 |
| CCN4/WISP1 | GGCGGCGGTAGGAGGAGATAC | CATAGGACCTTGCCGGGAGAGC | 498 |
| CCN5/WISP2 | ATGAGAGGCACAACCCAGAC | CTGGGCAAGGCCACAC | 425 |
| CCN6/WISP3 | GCCCGGCTGCCCCTCCTG | GGCAATTTTGTAGCTGTGAA | 414 |
of adult human buttock skin, and the mRNA levels were determined by quantitative real-time RT-PCR. Figure 1 shows that transcripts of all six CCN genes were detected in adult human skin. CCN5 was most abundantly expressed followed by CCN2>CCN3>CCN1>CCN4>CCN6. Expression of CCN5 transcript was several-fold higher than that observed for other CCN transcripts. mRNA expression of all six CCN genes was relatively low, compared to housekeeping gene, 36B4, and among them CCN6 was near the limit of detection.

**Regulation of CCN gene expression in human skin by UV irradiation** To investigate whether solar-simulated UV irradiation alters CCN gene expression, sun-protected human buttock skin was exposed to 2 MED (minimum erythema dose) solar-simulated UV irradiation. Skin samples were then taken at 4, 8, 16, 24, and 48 h following UV irradiation. Total RNA was prepared, and mRNA expression levels were determined by quantitative real-time RT-PCR. UV irradiation caused modest increases in both CCN1 and CCN2 at 4 and 8 h post UV irradiation (Fig. 2a, b). By 8 h, transcripts for both CCN1 and CCN2 were significantly increased approximately 15 and 4-fold, respectively. mRNA levels of both CCN1 and CCN2 returned to basal levels at 24 h following UV irradiation. In contrast, mRNA levels of CCN3, CCN4, CCN5, and CCN6 were modestly reduced following exposure of skin to UV irradiation. CCN3 mRNA levels decreased 40% at
24 h and returned to basal level at 48 h post UV irradiation (Fig. 2c). CCN4 mRNA levels were reduced 60% at 48 h following UV irradiation (Fig. 2d). CCN5 mRNA levels were decreased 50% at 24 h and returned to basal level at 48 h post UV irradiation (Fig. 2e). CCN6 mRNA levels were reduced 30% at 8 and 16 h and returned to basal level at 24 h post UV irradiation (Fig. 2f).

Discussion

Human skin protects the body from the deleterious environmental factor, including effects of UV-irradiation from the sun. Chronic exposures to UV irradiation over many years causes accumulative skin damage and eventually causes skin to age prematurely (photoaging) and leads to skin cancer (Fisher et al. 1996; Fisher et al. 1997; Yaar and Gilchrest 2007). Chronic sun exposure impairs normal skin functions, such as wound healing, immune surveillance, and skin barrier (Gilchrest and Yaar 1992; Kripke 1984; Pathak 1986; Pathak et al. 1993). Photodamaged skin also provides a microenvironment that supports the development of skin cancers, the most common type of cancer in the United States (Hales et al. 1989; Matsumura and Ananthaswamy 2002; Pilcher et al. 1997; Stamp et al. 1988). More than a million people are diagnosed with skin cancer each year and current estimates are that 40–50% of Americans who live to age 65 will have skin cancer at least once.

We found that all six CCN genes are expressed in human skin in vivo. Among them, CCN5 is most highly expressed followed by CCN2>CCN3>CCN1>CCN4>CCN6. In general, CCN1 and CCN2 are immediate early genes and are associated with cellular proliferation, whereas CCN3-5 are associated with suppression of proliferation (Bleau et al. 2007; Brigstock 1999; Delmolino et al. 2001; Huang et al. 2008; Kleer et al. 2007; Kleer et al. 2002; Perbal 2001; Planque and Perbal 2003; Zhang et al. 2005). Solar-simulated UV irradiation increased mRNA expression of proliferation-associated CCN genes, CCN1 and CCN2, at early time points in human skin in vivo. In contrast, mRNA expression of growth arrest-associated CCN genes, CCN3 CCN4, CCN5, and CCN6, were reduced by solar-simulated UV irradiation at late time points in human skin in vivo. Taken together, these mRNA expression data suggest that CCN protein may be involved in the hyperproliferative response that follows exposure of human skin to acute UV irradiation (Fisher and Voorhees 1998; Fisher et al. 1997; Matsumura and Ananthaswamy 2002; Quan et al. 2002b). Further investigations are required to determine cell-type specificity of CCN gene expression and localization of CCN proteins in human skin.

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