Ccn1, a molecular switch that imposes a self-limiting control on inflammation and wound healing in a multitude of organs?

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TO THE EDITOR: With great interest I have read the paper of Grazioli and colleagues that appeared in one of the April issues of this year (5). The findings that CCN1 expression increases in murine lung after bleomycin instillation and that the adenoviral transfer of CCN1 induces acute lung injury are of course very exciting. These observations strongly support our own experimental findings showing an increase in CCN1 expression during acute liver injury (2). In this former study, we have also found that the expression of CCN1 is strongly upregulated in activated hepatic stellate cells, whereas the expression decreases time dependently in these cells during transdifferentiation into fully differentiated myofibroblasts representing the key cellular subtype contributing to the progression of hepatic fibrogenesis. Moreover, we demonstrated that the adenoviral overexpression of CCN1 in mice induces production of reactive oxygen species leading to dose-dependent cellular senescence and apoptosis of liver cells (2). At that time our study was mainly inspired by the pioneering work of Lester F. Lau, who has established for the first time that CCN1 is a key player with a property to induce fibroblast senescence and restrict fibrosis in cutaneous wound healing (6). Moreover, Lau proved the ability of this matrix cell-adhesion molecule to trigger the formation and accumulation of a robust and sustained level of reactive oxygen species (7). So all these data obtained in skin, in liver, and now in lung might unanimously predict that CCN1 is a molecular switch that imposes a self-limiting control on inflammation and wound healing in a variety or potentially all organs. However, to prove the general validity of this assumption, it would be definitely necessary to clarify the reason(s) why Grazioli and coworkers failed to demonstrate the expression of the adenoviral-transduced CCN1 at the protein level.

The authors were only able to demonstrate a fivefold increase of ccn1/cyr61 expression in lung tissue after intratracheal delivery of AdCyr61. In my view, this slight increase in ccn1/cyr61 expression is somewhat unexpected since the transgene was expressed under transcriptional control of the strong constitutive active human cytomegalovirus (CMV) promoter. This might indicate an inadequate gene transfer, which could be improved by calcium phosphate precipitation of the adenoviral vector prior intratracheal intubation (4). Alternatively, the susceptibility of the different lung cells is restricted to certain cellular subsets of the pulmonary epithelium. The cellular ability to uptake adenoviral vectors could be simply tested in a histological stain for the Coxsackie adenovirus receptor (CAR) that is the common receptor for Coxsackie B viruses and adenoviruses type 2 and 5 (1). Another possibility to proof the efficacy of the adenoviral gene transfer would be to transduce an adenoviral expression vector driving the expression of the green fluorescent protein (GFP). Unfortunately, the authors provide insufficient information about the cloning and purification of the viruses that might impact the activity and toxicity (e.g., varying traces of caesium in individual preparations) of a "purified" virus preparation. They refer to an unknown entry vector (pENTR-1G) and a former paper (3) that, however, provide no additional information about the cloning of the viral vector or the exact purification protocol used to prepare the adenoviral particles for this study.

In addition, it would be mandatory to determine the reason(s) of the observed weight loss (10% not 90%) depicted in Fig. 3B that occurred 24 h after viral transfer. It would be highly interesting if the massive and rapid slim down after intratracheal instillation of the virus is due to lowered caloric intake, water deprivation, or malfunction of the digestive tract. Since the authors suggest that the measured weight reduction and increased mortality are due to an increase of lung injury, it would be further necessary to analyze whether the lung morphology was phenotypically altered and whether the measured increase in bronchoalveolar lavage fluid neutrophil counts impacts the total content of immune cells infiltrating the lung. It is quite possible that the weight loss is simply due to CCN1-induced promotion of epithelial cell death and tissue loss that was previously found in mice with emphysemaatous changes after prolonged cigarette smoke exposure (8). All these issues would be worth to be analyzed in future studies to understand the pathophysiological role of CCN1 in injured lung tissue.

At the end of my comments, I would like to take the opportunity to correct one mistake that has crept into the study of Grazioli and that perhaps will cause confusion to those readers that are not familiar with the CCN protein family nomenclature. The authors erroneously stated in the Abstract that the “Cysteine-rich protein-61 (CYR61), also known as connective tissue growth factor, CYR61, and nephroblastoma overexpressed gene 1 (CCN1), is a heparin-binding protein member of the CCN family of matricellular proteins.” Likewise, the authors mistakenly stated in the Introduction section “The cysteine-rich protein 61 (CYR61) (also known as connective tissue growth factor, CYR61, and nephroblastoma overexpressed gene 1, CCN1) is a 40-kDa heparin binding protein rich in cysteine residues.” According to the consensus guidelines of the International CCN Society that was supposed in the year 2003 (3), CCN1/CYR61, CCN2/CTGF, CCN3/
NOV, CCN4/WISP-1, CCN5/WISP-2, and CCN6/WISP-3 are different proteins that together form the family of CCN matricellular proteins.

GRANTS

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REFERENCES