processes of wound healing. Gross, histopathology, SEM and computer based 3D image probing techniques were utilized to quantify different toxic effects of MSWSS on wound healing. A total of 160 chicks, aged 1 week, divided in eight groups were exposed to MSWSS with different nicotine concentration; 0.2 mg (group A), 0.3 mg (group B), 0.5 mg (group C), 0 6 mg (group D), 0.7 mg (group E) and 1mg (group F). A very highly significant reduction ($P < 0.001$) in wound closure was observed among all MSWSS treated groups at day 8 post-wounding. Histological investigations revealed a significant impede outcome in the re-epithelializationof all MSWSS exposed wounds. Delayed dermal matrix regeneration and maturation of collagen bundles were observed among all MSWSS treated wounds. Similar results were achieved through SEM of treated wounds. Histological and image probing analysis unveiled the scanty neovascularizationamong MSWSS treated wounds. Abbot curve, angular spectrum and different other parameters of 3D surface topographiesof wounds revealed a very highly significant reduction ($P<0.001$) in angiogenesis among all MSWSS treated groups. These annotations validate the damaging effects of MSWSS on the healing of wounds.

Keywords Mainstream smoke, Cigarette, Chicken, Wound, Angiogenesis

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P#36

Functionally Active *Helicobacter Pylori* Vacuolating Cytotoxin in *Escherichia Coli*

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The vacuolating cytotoxin (VacA) which is a major pathogenic factor of *Helicobacter pylori* induces cellular vacuolation and apoptosis. Although the expression of this toxin in *Escherichia coli* has been attempted, the production of a functionally active recombinant VacA has been rare. In this study, we attempted to produce the active recombinant VacA with methionine substituted for alanine at N-terminal and 8X histidine tag conjugated at C-terminal in *E. coli* by inducing soluble expression at low temperature. We produced the 90-kDa VacA which was able to induce vacuolation and apoptosis in AGS cells and HeLa cells. In addition, anti-sera raised in a rabbit by intradermal injections of this recombinant proteins reacted in a immunoblot with a 88-kDa protein in supernatants from ATCC 49503 and ATCC 43504, *vacA* positive-strains with the *vacA* genotype s1/m1. Immunoglobulins purified by using protein G agarose neutralized the cytotoxic activity

induced by those supernatants. Separately, we assessed their potential as a diagnostic method to identify the presence of VacA by immunohistochemistry in both the liver tissue and the stomach tissue of patients with the corresponding cancer, respectively. These data indicate that this recombinant VacA has function and structure similar to those of native VacA, which could be useful in exploring the pathogenic role, especially correlation with hepatic diseases and developing a potential vaccine and a diagnostic kit.

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P#37

JNK1 Regulates α-Smooth Muscle Actin Expression by Transcriptional Activation and Ubiquitin-Dependent Degradation During Renal Fibrosis

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Myofibroblast, a specialized fibroblast characterized by expression of α-smooth muscle actin (α-SMA), play a major role in many tissue injuries and participate actively in the fibrotic process through extracellular matrix(ECM) production. Previously, we reported that phosphorylated form of c-Jun N-terminal kinase (JNK) was colocalized with α-SMA in myofibroblast during progressive renal disease. In the present study, to elucidate relationship between JNK and α-SMA in renal fibrosis, we investigated the kinetics of myofibroblast during the progression of renal fibrosis in rat using specific phosphorylated JNK (p-JNK) and α-SMA antibody, and elucidated regulatory mechanisms of α-SMA using wild type or deficiency fibroblast of JNK1 and JNK2. In a time-course in vivo study, a marked increase in p-JNK and α-SMA in both interstitial myofibroblast was shown in the progression stage of renal fibrosis, but decreased in end-stage renal fibrosis. Interestingly, overexpression of JNK1 and JNK2 reduced the expression of α-SMA gene, but increased α-SMA promoter activity in transfected cell line. While, JNK1-/- and JNK2-/- fibroblast increased the expression of α-SMA. Furthermore, we demonstrated that both the degradation and ubiquitination activity of α-SMA were more significantly increased by overexpression of JNK1 than JNK2. Collectively, our results suggest that α-SMA by JNKs is activated in the transcription level, but degradated in posttranslation level