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Detection of mRNAs for Keratinocyte Growth Factor and its receptor in human burned skin

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We examined the localization of Keratinocyte Growth Factor (KGF) and KGF Receptor (KGFR) on a human epithelium in a reproduction process after burning, mainly by using the non-radioactive In Situ Hybridization(ISH). We gained the regenerating epithelium reproduced around the damaged part from 24 burn cases. First, total RNA was extracted from the sample by using the AGPC and then the RT-PCR for KGF and KGFR mRNAs were performed. The PCR product was identified by confirming the band using 3% AGE. Second, regenerating epitheliums were immediately fixed in a 4% paraformaldehyde solution, and embedded in paraffin. After cut 3 um sliced tissues, ISH for KGF and KGFR mRNA were performed. We then created synthetic oligo-DNA by adding the ATT repeat to the end of the 45mer which is complementary to part of KGFmRNA and KGFRmRNA. Then we made T-T dimer by irradiating with UV light, used it as a probe, executed the ISH method, and detected the signal by enzyme immunoassay by using the HRP mark T-T dimmer antibody. Healing of the reproduced epithelium occurred from the surrounding part to the center of the damaged part. Histologically, the part could be classified into three phases:(1) A part having serious exudation, ulceration, and acute inflammation, (2) a part mainly consisting of incompletely reproduced epithelium and granulation, and (3) a part that was completely covered with reproduced epithelium (cicatrix cure). About the cases in which KGF and KGFRmRNA occurrence was confirmed using RT-PCR, their locations were confirmed using the ISH method. KGFmRNA was found in each of phases (1) to (3) above. Its occurrence was confirmed at many points in the fibroblast in the reproduced dermis. KGFRmRNA was found in some basal cells on the reproduced epithelium (in phase (2)), but was not found in the cicatrix cure tissue (phase (3)). KGFRmRNA occurrence was also found at a small number of points in phase (1). Therefore, it was proved that KGF and KGFR are the factors for causing reproduction. Especially, it was also proved that KGFR occurred transiently in the base cells of the reproduced epithelium in an early stage. We clearly recognized that the ISH method was useful in the examination of such local, transient gene manifestation.

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In Situ Hybridization of Human Renal Tissues

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Understanding the mechanisms of progression of renal diseases is important because of the tremendous increase in the number of patients with end-stage renal failure. Using digoxigenin-labeled oligonucleotide, we performed non-radioactive in situ hybridization for mRNAs associated with the development or progression of renal injury using human renal biopsy specimens. *Cytokines:* Using in situ hybridization for IL-4 and IL-6 mRNAs, we identified the expression of these cytokines at cellular level in glomerular-resident cells and interstitial cells. Comparison between results of immunohistochemistry for the same cytokines showed different expression patterns for the proteins and mRNAs in renal tissues, suggesting that in situ hybridization could detect the synthesis of cytokines that could not otherwise be detected by immunohistochemistry. *Complements:* Although complements are considered to be synthesized in the liver, recent studies have shown complement production in various other tissues. Results of positive staining for complement 3 (C3) in renal tissues by immunohistochemistry can not differentiate whether C3 is produced in the liver or renal tissues. In contrast, in situ hybridization for C3 mRNA clearly showed the renal production of C3 mRNA, as well as mRNA of the regulatory factor for C3 and clarified the association between the degree of renal tissue injury and renal synthesis of C3 and its regulatory factor. *Collagen:* We also examined the expression of mRNA of collagen type IV in human renal tissues. Since collagen turnover is slow, immunohistochemistry for collagen can show past but not current production of collagen. However, we were able to detect the production of collagen IV by in situ hybridization for collagen IV mRNA. *Semiquantitation of mRNA expression:* Since the level of retained RNA in the samples was variable, comparison of the degree of mRNA expression in different samples is not easy. We calculated the ratio between the expression of a specific mRNA to that of 28S rRNA using image analysis software and succeeded in comparing the level of expression of a specific mRNA in biopsy specimens with different levels of the retained RNA. In conclusion, in situ hybridization of renal biopsy specimens provides better information on gene expression, related to the progression of renal tissue injury, than other analytical methods. In situ hybridization using clinical samples may allow further evaluation of the pathogenesis of human diseases.