Multiple gene differential expression patterns in human ischemic liver: Safe limit of warm ischemic time

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AIM: To investigate the multiple gene differential expression patterns in human ischemic liver and to produce the evidence about the hepatic ischemic safety time.

METHODS: The responses of cells to hepatic ischemia and hypoxia at hepatic ischemia were analyzed by cDNA microarray representing 4,000 different human genes containing 200 apoptotic correlative genes.

RESULTS: There were lower or normal expression levels of apoptotic correlative genes during the periods of hepatic ischemia for 0-15 min, the maintenance homostatic genes were expressed significantly higher at the same time. But at the hepatic ischemia for 30 min, the expression levels of maintenance homeostatic genes were down-regulated, the expressions of many apoptotic correlative genes and nuclear transcription factors were activated and up-regulated.

CONCLUSION: HIF-1, APAF-1, PCDC10, FBX5, DFF40, DFFA XIAP, survivin may be regarded as the signal genes to judge the degree of hepatic ischemia-hypoxic injury, and the apoptotic liver cell injury due to ischemia in different time limits. The safe limit of human hepatic warm ischemic time appears to be generally less then 30 min.

Lu QP, Cao TJ, Zhang ZY, Liu W. Multiple gene differential expression patterns in human ischemic liver: Safe limit of warm ischemic time. World J Gastroenterol 2004; 10(14): 2130-2133

http://www.wjgnet.com/1007-9327/10/2130.asp
RESULTS

Comparison of differential gene expressions between 15 min ischemic group and normal control group

Figure 1 (A and B) shows that the double-colored fluorescent labels in 15 min ischemic group and normal control group were overlapping. A total of 41 differentially expressed genes were identified by cDNA chip between the 15 min ischemic group and normal control group. Among them, the ratio values of 33 genes were >2, of which 7 were the significantly elevated, the ratio values of 4 genes were >4, and 20 genes only expressed the rising tendency but their ratio values were >2. In the meantime, there were 6 differential expression genes whose ratio values were >2, of which 1 gene was correlated directly with cell apoptosis regulation (the congenic gene of survivin, AB028869 and X-linked inhibitor apoptosis protein, u45880 respectively), and 1 congenic gene (GenBank-ID: af004711) of TPKC1. In the meantime, there were 6 differential expression genes whose ratio values were >2, of which 3 were the regulation genes correlated directly with cell apoptosis: APAF1 (GenBank-ID: NM-001160), PDCD10 (GenBank-ID: NM-007210) and an unnamed gene (GenBank-ID: AL031714), 1 was the congenic gene (GenBank-ID: AF050127) of HIF1, the functions of last 2 genes’ were not clear.

Comparison of differential gene expressions between 30 min ischemic group and normal control group

In the 200 genes, 35 genes were not expressed at the two time limits. The expressions of 167 genes in 30 min ischemic group differed from in 15 min ischemic group. One hundred and nineteen genes were expressed following the rising tendency, of which ratio values of 7 genes were >5, 19 >3, 60 000, and 55 genes only expressed the rising tendency but their ratio values were ≤++. 46 genes were following the descending tendency, of which ratio values of 4 genes were >1.5, 5 >1.5, 12 >1.5, and 25 genes only expressed the descending tendency but their ratio values were ≤1.5. In addition, 16 genes were expressed in 30 min ischemic group, but not in 15 min ischemic group, of which 11 genes were

Figure 1 Double-colored fluorescent labels and scattered diagram of signal intensity of differential gene expressions in 15 min ischemic group and normal control group. Green: Down-regulation, Signal of cy3 is stronger; Red: Up-regulation, signal of cy5 is stronger. A, C: Double-colored fluorescent labels: In 15 min ischemic group and normal control group; B, D: Scatter diagram of signal intensity of differential gene expressions in 15 min ischemia group and normal control group.
correlated directly with cell apoptosis regulation, 5 genes were expressed in 15 min ischemic group, but not in 30 min ischemic group, of which only 1 gene was correlated directly with cell apoptosis regulation, namely XIAP (GenBank-ID: u45880).

**DISCUSSION**

Our data suggested that the apoptosis regulation gene at the hepatic ischemia for 0-30 min presented following features.

In hepatic ischemia for 0-15 min, the apoptosis regulation gene was expressed low or normally. The differentially expressed genes whose ratio values were >2 (namely, they were expressed following the rising tendency) were mainly the maintenance harmonious genes, such as genes regulate P43=mitochondrial elongation factor homolog (GenBank-ID: S75463), Human ribosomal protein S14 gene (GenBank-ID: huRMPs), H. sapiens mRNA for squalene synthase (GenBank-ID: hssqsyn), Na+/K+-ATPase activity c subunit (GenBank-ID: hsatpar) and β subunit (GenBank-ID: humatpbi), H+-ATPase activity (GenBank-ID: hsatp). But they were not highly expressed with the congenic genes correlated directly with cell apoptosis regulation.

In hepatic ischemia for 15-30 min, the expression of apoptosis regulation genes changed greatly. One hundred and nineteen genes were expressed following the rising tendency, of which 59 genes were relevant to the apoptosis regulation. Although some anti-apoptosis genes [such as the congenic gene of bcl-2 (GenBank-ID: AJ006288), the congenic gene of inhibitor apoptosis protein IEX-IL (GenBank-ID: AF071596)] which were expressed the low level, the majority of the higher expressed genes were the apoptotic genes, for example, the congenic gene of apoptotic protease activating factor-1 (APAF-1, GenBank-ID: NM-001160); Homo sapiens F-box protein 5, FBX5 (GenBank-ID: NM-012177); DNA fragmentation factor, 40 ku, subunit, DFF40 (GenBank-ID: AF064019); DNA fragmentation factor, 45 ku, alpha subunit, DFFA (GenBank-ID: NM-004401); p53-induced protein; PIG11 (GenBank-ID: NM-006034).

Especially, APAF-1 was the only known human homologue of CED-4 and the nuclear element in the formation of apoptosis body.[13-16] Now it is thought as the key element regulating cell apoptosis, and the ratio value of its expression in 30 min hepatic ischemia was an additional “+++” than that in 15 min hepatic ischemia. Meanwhile the ratio value of the congenic gene of PDCD10 (GenBank-ID: NM-007217) was also “+++”. The significant rising of expression of the apoptotic genes showed that the unavoidable cell death after serious ischemic injury was a key expression of the serious cell injury. In 15 min hepatic ischemia, the congenic gene of the important X-linked inhibitor apoptosis protein, XIAP (GenBank-ID: u45880) changed from the normal expression to non-expression, following the obvious descending tendency. Compared with the normal group, the expression of the survivin congenic gene (GenBank-ID: AB028869) descended greatly with the ratio value <0.5. The codogenic genes (GenBank-ID: S75463, humrps, hssqsyn, hsatpar, humatpbi, hsatp) and so on) related to the internal circumstance and the organelle functions were expressed obviously in the period of 0-15 min hepatic ischemia, but in 15-30 min period their expression descended to “+++”. The congenic gene of human hypoxia-inducible factor 1 HIF-1 alpha gene (GenBank-ID: AF050127) was expressed apparently (increased to “+++”). Lots of the regulation genes of nucleus translation factors were expressed notably for example, the codogenic gene of EIF4G2 (eukaryotic translation-initiation factor 4 gamma, 2 (EIF4G2) mRNA, GenBank-ID: NM-001418), NF-κB family, TNF receptor associated factor 6 (TRAF6) responsible for the existence and death of cells (GenBank-ID: hsu78798), increased to “+++”. The latter was a transmission factor participating in IL-1 signal transmission and activating the signal transmission about apoptosis of the nuclear factor NF-κB.[17-20]

The data suggested that in 0-15 min hepatic ischemia, the gene regulation model inside of the cells was likely to better keep the wholeness of cell and organ functions even by controlling ion channel and organelle functions. At the same time, although acute ischemic-hypoxic injury changed the structure and functions of cells, the expression of each kind of genes relevant to apoptosis, according to the analysis of the level of gene regulation, remained on the low level, that is to say, the occurrence of apoptosis was not a main direction yet. With the time of ischemia and hypoxia passed, when hepatic ischemia lasted for 30 min, acute ischemic-hypoxic injury perhaps would lead to the significant rising of HIF-1, and the cell gene expression model responsible for the existence or death of the cells also was changed completely. The expressions of all kinds of regulation genes maintaining cells and the internal cellular circumstance descended obviously, while NF-κB and many genes relevant to apoptosis were activated evidently and notably expressed. Therefore, it is suggested that cell death is unavoidable when hepatic ischemia lasts for 30 min. It is true that some experts deduced that the time of the human hepatic ischemia could be prolonged beyond the safe limit according to clinical experience in the progress after liver operation. Yet when dynamic changes of gene expressions after liver suffered ischemic injury are analyzed, it is suggested that the safe limit of one hepatic ischemia should be less than 30 min, namely, when key death genes such as APAF-1, PDCD10, FBX5, DFF40, DFFA are activated evidently and expressed significantly, and before the obvious descending of inhibitor apoptosis genes such as XIAP, survivin. In the meantime it is suggested that HIF-1, APAF-1, PDCD10, XIAP, survivin may be regarded as the signal gene to judge the degree of hepatic ischemic-hypoxic injury, and the apoptotic liver cell injury due to ischemia in different time limits.

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Edited by Wang XL. Proofread by Chen WW and Xu FM