Laboratory Investigation (2005) 85, 836–837

www.laboratoryinvestigation.org

Pathology Elsewhere

Laboratory Investigation (2005) **85,** 836–837.
doi:10.1038/labinvest.3700297

Defining the toll-like receptor (TLR): type-1 interferon (IFN) pathway in liver disease

Toll-like receptors (TLR) play a crucial role in various inflammatory diseases. TLR constitute the first step in innate immunity by recognizing structurally conserved bacterial and viral components. TLR play a role in the induction of acquired immunity through their involvement in maturation of dendritic cells and by driving a Th1 cell response. Binding of pathogen-specific ligands to TLR in early infection leads to expression of multiple protective cellular genes. The induction of the protective type-1 IFN genes by the activated TLR is mediated by a transcription factor—IFN regulatory factor 3 (IRF-3). Hepatitis C virus (HCV), notorious for its ability to establish persistent infection, is among many viruses that have evolved strategies to block these signaling pathways. Activation of IRF-3 has previously been shown to limit the replication of HCV RNA in cultured hepatocytes, while disruption of these signaling pathways by NS3/4A, an HCV protease, leads to viral persistence. Other protective genes may be involved, but most of these have not been clearly defined.

In a recent article, **Li *et al***[1] showed that NS3/4A causes specific proteolysis of an adaptor protein (TRIF) linking TLR3 to kinases responsible for activating IRF-3 and NF-κB, which consequently blocked the induction of type-1 IFN genes. The cleavage of TRIF by NS3/4A represented a more proximal attack on the pathway before its bifurcation to IRF-3 and NF-κB. This early proteolysis of TRIF inhibited polyI:C-activated signaling through the TLR3 pathway, thereby potentially limiting expression of multiple host defense genes in HCV infection. The authors also concluded that inhibition of the NS3/4A protease by antiviral agents might not only suppress viral replication, but also restore innate antiviral defenses to the virus-infected hepatocytes.

In this issue of *Lab Invest*, **Takii *et al***[2] demonstrated the importance of an activated TLR3-type-1 IFN signaling pathways in sustaining portal and lobular inflammation in early primary biliary cirrhosis. Type-1 IFN and TLR-3 proteins demonstrable by immunohistochemistry in the portal tracts and liver parenchyma in primary biliary cirrhosis were not observed in chronic hepatitis C and autoimmune hepatitis.

Both studies demonstrate the importance of type-1 IFN induction via the TLR signaling pathways in the inflammatory response of inflammatory liver diseases. Persistent HCV infection occurs when the IFN-signaling pathways are blocked, whereas primary biliary cirrhosis arises in the setting of sustained activation of IFN signaling. Clearly, while inflammatory states in chronic liver disease are critically affected by regulation of IFN signaling pathways, the causal relationships may be quite divergent.

Maria Isabel Fiel, MD

References

1 Li K, Foy E, Ferreon JC, *et al*. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the toll-like receptor 3 adaptor protein TRIF. Proc Natl Acad Sci 2005;102:2992–2997.
2 Takii Y, Nakamura M, Ito M, *et al*. Enhanced expression of type 1 interferon and toll-like receptor-3 in primary biliary cirrhosis. Lab Invest 2005;85:908–920.

Gene amplification patterns in human tumorigenesis

The DNA changes that result in tumor formation are highly complex. Genes that relate to tumorigenesis are often divided into oncogenes and tumor suppressor genes. Gene amplification is often necessary for oncogenes to cause tumor formation. The elucidation of these DNA aberrations requires molecular analyses with such techniques as tissue microarrays and fluorescent *in situ* hybridization (FISH). FISH is based on the complementary double-stranded nature of DNA and the hybridization of specific, fluorescently labeled, DNA-probes. Owing to the relative stability of DNA, determination of gene amplification is possible on archival tissue, making this technique very practical for most practicing pathology departments. Tissue microarrays allow use of one probe to simultaneously evaluate small, representative samples of numerous tumors placed on one slide.

In a recent study, **Al-Kuraya *et al***[1] used these techniques to evaluate the amplification pattern of five oncogenes in human breast cancer; HER2, EGFR, MYC, CCND1, and MDM2, and correlated the findings with the clinical follow-up information. The authors created six tissue microarray (TMA) blocks, each of which contained between 342 and 522 'punched out', representative 0.6 mm tissue cylinders. Sections of the TMA blocks were probed for specific genes, using the respective chromosomal centromere regions as a reference. A gene was considered amplified if the ratio of oncogene/centromere was ≥ 2.0. The frequency of the gene amplifications was 17.3% for HER2, 0.8% for

EGFR, 5.3% for MYC, 20.1% for CCND1, and 5.7% for MDM2. All gene amplifications were strongly associated with high tumor grade, while only HER2 amplification was shown to be an independent variable in predicting poor patient outcome. MYC amplifications were almost three times more frequent in medullary cancers. There was also a strong link of HER2 and MYC amplifications to ER/PR negativity. Coamplifications of genes occurred more frequently than expected based on their individual frequencies, suggesting an overall genetic instability among malignant cells. These findings confirmed many other studies regarding HER2 and provided an additional insight on MYC and the genetic instability of breast carcinomas.

Using very similar methodologies and target genes, **Tornillo *et al***,[2] in this issue of *Lab Invest*, evaluated gastrointestinal stromal tumor gene amplification patterns. These authors found that amplifications were seen in 22% of malignant tumors, nearly 50% of which were MDM2 and CCND1. These studies demonstrate that gene-specific probes applied to large sample sets obtained by tissue microarray, are an effective tool for unlocking the genetic profiles of human neoplasia.

Gabriel Levi, MD

References

1 Al-Kuraya K, Schraml P, Torhorst J, *et al*. Prognostic relevance of gene amplifications and coamplifications in breast cancer. Cancer Res 2004;64:8534–8540.
2 Tornillo L, Duchini G, Carafa V, *et al*. Patterns of gene amplification in gastrointestinal stromal tumors (GIST). Lab Invest 2005;85:921–931.

NF-κB pathway provides a link between HTLV-1 Tax and survivin

Human T-lymphotropic virus (HTLV) is the etiologic agent of adult T-cell leukemia (ATL). Its viral protein Tax plays an important role in the transformation and immortalization of leukemic cells, and appears to protect leukemic cells from apoptosis. Tax transactivates many cellular genes through modulation of host transcription factors, among which is NF-κB. Activation of NF-κB is detected in a wide variety of human cancer cells and is often associated with cellular transformation by viral oncoproteins. On the other hand, survivin, the smallest member of the inhibitor-of-apoptosis (IAP) gene family, is uniquely overexpressed in many cancers and plays a more selective role than other IAPs in antagonizing mitochondrial-dependent apoptosis. Freshly obtained leukemic cells from patients with ATL and HTLV-I-infected T-cell lines are known to express high levels of survivin. In addition, high survivin transcript levels correlate significantly with poor outcome in ATL patients.

Although survivin expression is detected in various cancers, regulation of its expression remains largely unknown. In a recently published study, **Kawakami *et al***[1] investigated the transcriptional regulation of survivin in HTLV-I-infected and Tax-expressing cells. They had previously found that resistance to apoptosis in Tax-expressing cells is induced by the absence of IL-2. In this study, the expression of survivin in Tax-expressing cells was associated with resistance to apoptosis after IL-2 deprivation. Furthermore, the NF-κB pathway is required for the full activation of survivin promoter by Tax. Based on these findings, they concluded that Tax and IL-2 regulate the expression of survivin through NF-κB pathway.

In this issue of *Lab Invest*, **Tsuji *et al***[2] showed that malignant transformation of thymomas could be induced by heterotopic transplantation of thymuses from newborn transgenic rats expressing the pX gene of HTLV-1 (known to encode Tax protein) into other thymectomized transgenic rats. The transformed tumors appeared to express less of the pX gene and undergo deletion of the p16 gene, which was otherwise upregulated in the nontransformed thymomas. This suggests that the aggressive form of neoplastic cells may not require the expression of pX gene any further, once they have reached a certain state of alteration of host genes. Both studies provide new insights for understanding the mechanism of HTLV-1 induced malignancies and possible targeted treatment of ATL.

Arief Suriawinata, MD

References

1 Kawakami H, Tomita M, Matsuda T, *et al*. Transcriptional activation of survivin through the NF-kappa B pathway by human T-cell leukemia virus type 1 Tax. Int J Cancer 2005;Feb 23, Epub ahead of print.
2 Tsuji T, Ikeda H, Tsuchikawa T, *et al*. Malignant transformation of thymoma in recipient rats by heterotopic thymus transplantation from HTLV-1 transgenic rats. Lab Invest 2005;85:851–861.