complexes and releases bound Cip/Kip proteins. Agents acting "upstream" (such as inhibitors of Ras, Raf, and PI3 kinases) can prevent the induction of cyclin D1, limit its stability, or interfere with its assembly with Cdks, and these might prove efficacious. The ultimate goal must be to translate these important new molecular insights into more effective treatment of MCL; until then, we will continue to measure success as extended survival and not cure.

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Selected reading

Barista, I., Romaguera, J.E., and Cabanillas, F. (2001). Lancet Oncol. *2*, 141–148.

Hofmann, W.K., de Vos, S., Tsukasaki, K., Wachsman, W., Pinkus, G.S., Said, J.W., and Koeffler, H.P. (2001). Blood *98*, 787–794.

Khouri, I.F., Romaguera, J., Kantarjian, H., Palmer, J.L., Pugh, W.C., Korbling, M., Hagemeister, F., Samuels, B., Rodriguez, A., Giralt, S., et al. (1998). J. Clin. Oncol. *16*, 3803–3809.

Rosenwald, A., Wright, G., Wiestner, A., Chan, W.C., Connors, J.M., Campo, E., and Staudt, L.M. (2003). Cancer Cell *3*, this issue, 185–197.

Senderowicz, A.M. (2001). Leukemia *15*, 1–9.

Schmitt, C.A., Fridman, J.S., Yang, M., Lee, S., Baranov, E., Hoffman, R.M., and Lowe, S.W. (2002). Cell *109*, 335–346.

Sherr, C.J., and Roberts, J.N. (1999). Genes Dev. *13*, 1501–1512.

Oncogene at last—c-Jun promotes liver cancer in mice

c-Jun, a component of transcription factor AP-1, has been known to play an important role in the control of cell proliferation. It was also suspected to be a critical mediator of tumor promotion. In a recent paper in Cell, Eferl et al. have now provided conclusive evidence that c-Jun expression is critical for induction of liver cancer by a classical protocol of tumor initiation—tumor promotion.

The c-Jun protein is a component of transcription factor AP-1 (Angel and Karin, 1991), encoded by the *c-jun* gene (using mouse gene terminology), the cellular homolog of the retroviral *v-jun* oncogene (Vogt, 2001). The discovery fifteen years ago that c-Jun together with c-Fos is a component of AP-1, a transcription factor implicated in the induction of gene transcription by phorbol ester tumor promoters (Angel et al., 1987), generated a great deal of excitement at the time. For once it suggested a biochemical function for c-Jun being one of the first sequence-specific transcription factors found to be encoded by a proto-oncogene. Even more importantly, it suggested that the putative pro-oncogenic function of c-Jun is due to its function at the receiving end of a signal transduction pathway that mediates gene induction by phorbol esters and other tumor promoters. This discovery also provided a molecular mechanism and an explanation for tumor promotion, suggesting that tumor promoters are chemical and physical agents that can activate signaling pathways that stimulate the activity of transcription factors that regulate the expression of genes involved in cell proliferation and neoplastic trasformation. This hypothesis implicated that chronic elevation of c-Jun's expression or activity as brought about by tumor promoters should lead to oncogenic transformation. However, direct genetic evidence in favor of this hypothesis has been lacking. Unlike other mammalian proto-oncogenes, mutations in the *c-jun* locus have not been found in human or murine cancers and overexpression of the normal c-Jun protein does not readily result in transformation of rodent fibroblasts (Shaulian and Karin, 2002). This important deficiency has finally been rectified. Eferl et al. report in the recent issue of *Cell* that a targeted disruption of the *c-jun* gene in mouse hepatocytes does not interfere with normal function, but prevents the emergence of hepatocellular carcinomas in response to a classical model of tumor initiation-tumor promotion (Eferl et al., 2003). These results not only prove that c-Jun is a critical component of the carcinogenic mechanism but also suggest that c-Jun antagonists may be used in chemoprevention of liver cancer, a significant health problem in certain parts of the world.

The acute or chronic loss of hepatic function caused by alcohol, viral infection, or other hepatotoxic drugs can result in severe illness such as fulminant hepatitis, or cirrhosis, and greatly increases the risk for eventual development of hepatocellular carcinoma (Okuda, 2000). Chronic infections with the hepatitis B virus (HBV) and the hepatitis C virus (HCV) represent major risk factors for hepatocellular carcinoma (Okuda, 2000). AP-1 was reported to be activated in both hepatocellular carcinoma and chronic hepatitis (Liu et al., 2002). In vitro studies using liver-derived cell lines have demonstrated rapid activation of AP-1 by HBV or HCV proteins (Kato et al., 2000). Thus, there had been ample reasons to suspect the involvement of c-Jun or other AP-1 proteins in liver cancer.

In addition to c-Jun and c-Fos, AP-1 transcription factors are composed of homo- and heterodimers of basic region-leucine zipper (bZIP) proteins that belong to the Jun (c-Jun, JunB, and JunD) and Fos (c-Fos, FosB, Fra-1, and Fra-2) subfamilies, all of which recognize the AP-1

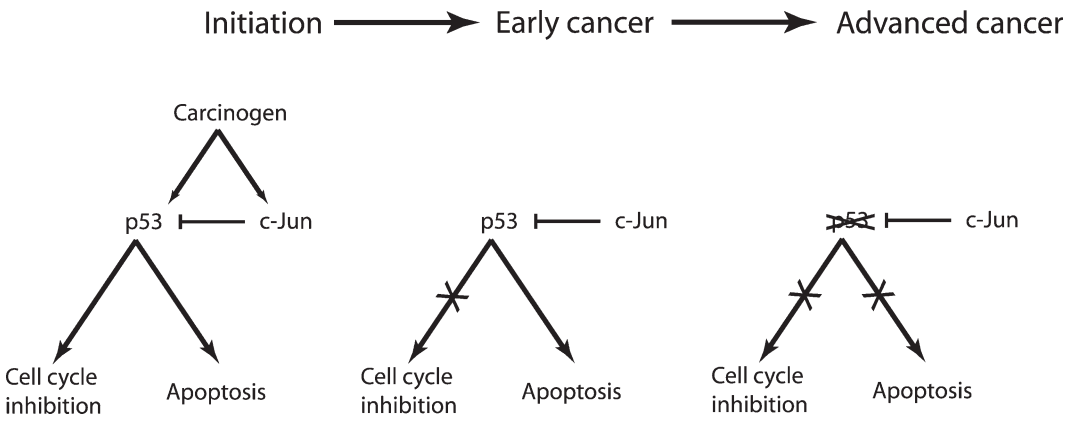

Figure 1. Putative scenario of c-Jun function in tumor development

When normal liver cells are exposed to a tumor initiator (carcinogen), both c-Jun and p53 are induced and c-Jun can antagonize the pro-apoptotic and anti-proliferative activities of p53 (initiation stage). Once cells are transformed, they may become insensitive to the anti-proliferative activity of p53 (early cancer stage). In advanced stages of cancer, most of the tumor cells have incurred p53 gene mutations rendering p53 nonfunctional, so c-Jun cannot affect p53 function at this stage.

binding site or TPA response element (TRE) in the regulatory region of AP-1 target genes (Angel and Karin, 1991; Shaulian and Karin, 2002). These proteins also can heterodimerize with other transcription factors such as members of the ATF/CREB and Maf/Nrl subfamilies (Shaulian and Karin, 2002). In addition to phorbol ester tumor promoters, such as TPA, AP-1 transcription factors are activated by many physiological and pathophysiological stimuli including growth factors, oncoproteins such as Src and Ras, proinflammatory cytokines, and UV radiation (Angel and Karin, 1991). Many of these stimuli can function as tumor promoters or are known to activate oncogenic signaling cascades. Amongst the AP-1 proteins, c-Jun seems to be most important for stimulation of cell proliferation (Shaulian and Karin, 2002). Mouse fibroblasts lacking c-Jun exhibit a severe proliferation defect mostly due to a dramatic extension of their G1 transition time (Schreiber et al., 1999). When exposed to UV radiation, these cells do not undergo apoptosis but exhibit premature senescence and reduced ability to re-enter the cell cycle (Shaulian et al., 2000). Fibroblasts deficient in other Jun or Fos proteins do not exhibit such defects, and there is even evidence that JunD may be a negative regulator of cell proliferation (Shaulian and Karin, 2002). Interestingly, the defective proliferation of c-Jun-deficient cells has been attributed to elevated expression of p53 and its target gene *p21^{waf1}* (Schreiber et al., 1999; Shaulian et al., 2000). Although overexpression of c-Jun is usually not sufficient for transformation of rodent fibroblasts, when coexpressed with the oncogenic Ha-Ras (V12) protein, c-Jun does augment Ras-mediated cell transformation (Binétruy et al., 1991). This activity is not exhibited by JunB or JunD and is dependent on the N-terminal phosphorylation of c-Jun by Jun kinases (JNKs) (Smeal et al., 1991). Furthermore, mouse fibroblasts lacking c-Jun are refractory to transformation by oncogenes, such as *Ha-ras* or *v-src* (Johnson et al., 1996). Thus, there has been considerable evidence that in cultured fibroblasts, c-Jun is required for proper cell proliferation and for oncogenic transformation. However, the evidence for c-Jun's involvement in tumor formation in vivo has been lacking, until now.

c-Jun-deficient mice die between embryonic days E12.5 and E13.5 from massive apoptosis of hepatoblasts, erythroblasts, and other cell types (Hilberg et al., 1993; Johnson et al., 1993). To overcome this problem, mice harboring a "floxed" *c-jun* allele that can be deleted in designated cell types upon expression of the Cre recombinase have been developed. Using this system, the Wagner lab has found that c-Jun expression is required for proper proliferation in postnatal hepatocytes (Behrens et al., 2002). Moreover, the deletion of *c-jun* in hepatocytes does compromise the ability of these cells to enter the cell cycle and undergo rapid proliferation after partial hepatectomy (Behrens et al., 2002).

Using mice lacking c-Jun in hepatocytes (so called *c-jun$^{\Delta li}$* mice), Eferl et al. have examined the role of c-Jun in liver carcinogenesis (Eferl et al., 2003). They employed a well-established model of chemical carcinogenesis based on the tumor initiator diethylnitrosamine (DEN) and the tumor promoter phenobarbital (Kato et al., 1993). The results were clear-cut; liver tumor mass was dramatically reduced and survival rate was notably improved in *c-jun$^{\Delta li}$* mice in comparison to the "floxed" mice (*c-jun$^{F/F}$*) used as controls. Eferl et al. investigated the mechanism accounting for the reduced tumorigenic effect and found that apoptosis was increased in liver tumor cells originating from *c-jun$^{\Delta li}$* mice, whereas the proliferation of these cells was not reduced in comparison to tumor cells derived from *c-jun$^{F/F}$* mice. These results seem unexpected since earlier work from the same group has shown that c-Jun is required for optimal hepatocyte proliferation (Behrens et al., 2002). Most likely, those tumors that form in *c-jun$^{\Delta li}$* mice have found ways to achieve high rates of cell proliferation even in c-Jun's absence. Eferl et al. used inducible Cre expression to determine at which stage of liver tumor development c-Jun was required. The results suggested to them that c-Jun is required in the initiation stage of liver carcinogenesis but not during progression. However, the particular experimental design used did not allow clear-cut evaluation of whether c-Jun is involved in tumor promotion.

Based on a protocol in which tumors were induced by administration of DEN alone in the absence of phenobarbital, Eferl et al. conclude that c-Jun is required for initiation and not promotion. Yet, in another experiment, they deleted c-Jun after tumor initiation and found reduced tumor incidence. Most likely, c-Jun is required for the survival of initiated cells and thus is needed for tumor promoter action, whose major function is the increased survival and expansion of initiated cells. As the role of c-Jun in DNA repair or the effect of its deletion on formation of mutagenic lesions in response to DEN has not been evaluated, it is difficult to conclude that c-Jun is required for tumor initiation per se.

How can c-Jun prevent apoptosis? Eferl et al. analyzed the anti-apoptotic function of c-Jun using a cell culture system and found that c-Jun-deficient hepatocytes are more sensitive to TNFα-induced apoptosis and that this sensitization was rescued by a p53 deficiency. They also found that a p53-regulated pro-apoptotic gene, *Noxa*, was upregulated in c-Jun-deficient tumors. These results suggest that p53 may be required for TNFα-induced apoptosis. However, until now, TNFα-induced apoptosis has never been shown to depend on p53 activation and TNFα was not known as a p53 activator. Yet, the connection between c-Jun and p53, as described above, is certainly not surprising. c-Jun-deficient fibroblasts exhibit elevated expression of p53 and its target gene *p21^{waf1}*, which encodes an inhibitor of cyclin-dependent kinases (Schreiber et al., 1999). Indeed, the elevated expression of p53 and p21^{waf1} was shown to be the major cause of the proliferation defect of these cells. Elevated p53 and p21^{waf1} expression are also responsible for the extended UV-induced growth arrest in *c-jun*$^{-/-}$ fibroblasts (Shaulian et al., 2000). Thus the important question is how c-Jun controls p53 expression and transcriptional activity. Although this mechanism remains a mystery, Eferl et al. show that c-Jun-deficient liver tumors accumulate high levels of p53 protein, just like c-Jun-deficient fibroblasts. Curiously, however, elevated levels of p53 could not be detected in c-Jun-deficient hepatocytes.

Apoptosis is a physiological mechanism that eliminates undesired cells during development, immune responses, and oncogenic transformation. c-Jun appears to be both a positive and a negative regulator of apoptosis (Shaulian and Karin, 2002). The exact function of c-Jun is likely to be cell type and stimulus specific. For example, inhibition of c-Jun activity protects sympathetic neurons against NGF withdrawal-induced apoptosis (Ham et al., 1995). Mice expressing a c-Jun with alanines at positions 63 and 73 (c-JunA63/73), which can no longer be phosphorylated by JNK, are resistant to kainate-mediated neuronal apoptosis (Behrens et al., 1999). In contrast to these results, the liver *of c-jun*$^{-/-}$ fetal mice exhibits elevated numbers of apoptotic cells, suggesting that c-Jun has an anti-apoptotic function (Eferl et al., 1999). This anti-apoptotic activity does not seem to depend on JNK-mediated phosphorylation, as mice expressing c-Jun (A63/73) are fully susceptible to liver carcinogenesis (Eferl et al., 2003). Rather, the anti-apoptotic function of c-Jun may be related to its ability to reduce p53 accumulation (Schreiber et al., 1999) and inhibit p53 binding to the *p21^{waf1}* promoter (Shaulian et al., 2000). Thus, the intriguing antagonism between c-Jun and p53, a major tumor suppressor, may underlie the tumor-promoting activity of c-Jun (Figure 1). When normal liver cells are exposed to a tumor initiator like DEN, which is a potent genotoxic agent, both c-Jun and p53 are induced and c-Jun can antagonize the pro-apoptotic and anti-proliferative activities of p53 (Figure 1). Thus, c-Jun-deficient cells would be eliminated more readily than wild-type cells through p53-mediated apoptosis. This accounts for reduced tumor incidence in the absence of c-Jun. However, once wild-type or *c-jun*$^{-/-}$ cells are transformed, they may become insensitive to the anti-proliferative activity of p53 (Figure 1). In advanced stages of cancer, most of the tumor cells have incurred *p53* gene mutations rendering p53 nonfunctional, so c-Jun cannot affect p53 function at this stage. Based on this scheme, c-Jun inhibitors may be particularly useful during pre-cancerous states like cirrhosis or chronic viral infection, as chemopreventive agents, but rather useless in the therapy of advanced *p53*-mutated tumors (Figure 1). The design of c-Jun inhibitors should be therefore seriously considered and contemplated. The availability of such inhibitors may further clarify how c-Jun acts to promote liver cancer and possibly other forms of cancer as well.

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Selected reading

Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R.J., Rahmsdorf, H.J., Jonat, C., Herrlich, P., and Karin, M. (1987). Cell *49*, 729–739.

Angel, P., and Karin, M. (1991). Biochem. Biophys. Acta *1072*, 129–157.

Behrens, A., Sibilia, M., David, J.P., Mohle-Steinlein, U., Tronche, F., Schutz, G., and Wagner, E.F. (2002). EMBO J. *21*, 1782–1790.

Behrens, A., Sibilia, M., and Wagner, E.F. (1999). Nat. Genet. *21*, 326–329.

Binétruy, B., Smeal, T., and Karin, M. (1991). Nature *351*, 122–127.

Eferl, R., Ricci, R., Kenner, L., Zenz, R., David, J.P., Rath, M., and Wagner, E.F. (2003). Cell *112*, 181–192.

Eferl, R., Sibilia, M., Hilberg, F., Fuchsbichler, A., Kufferath, I., Guertl, B., Zenz, R., Wagner, E.F., and Zatloukal, K. (1999). J. Cell Biol. *145*, 1049–1061.

Ham, J., Babij, C., Whitfield, J., Pfarr, C.M., Lallemand, D., Yaniv, M., and Rubin, L.L. (1995). Neuron *14*, 927–939.

Hilberg, F., Aguzzi, A., Howells, N., and Wagner, E.F. (1993). Nature *365*, 179–181.

Johnson, R., Spiegelman, B., Hanahan, D., and Wisdom, R. (1996). Mol. Cell. Biol. *16*, 4504–4511.

Johnson, R.S., van Lingen, B., Papaioannou, V.E., and Spiegelman, B.W. (1993). Genes Dev. *7*, 1309–1317.

Kato, M., Popp, J.A., Conolly, R.B., and Cattley, R.C. (1993). Fundam. Appl. Toxicol. *20*, 155–162.

Kato, N., Yoshida, H., Kioko Ono-Nita, S., Kato, J., Goto, T., Otsuka, M., Lan, K., Matsushima, K., Shiratori, Y., and Omata, M. (2000). Hepatology *32*, 405–412.

Liu, P., Kimmoun, E., Legrand, A., Sauvanet, A., Degott, C., Lardeux, B., and Bernuau, D. (2002). J. Hepatol. *37*, 63–71.

Okuda, K. (2000). J. Hepatol. *32* (*Suppl 1*), 225–237.

Schreiber, M., Kolbus, A., Piu, F., Szabowski, A., Möhle-Steinlein, U., Tian, J.M., Karin, M., Angel, P., and Wagner, E.F. (1999). Genes Dev. *13*, 607–619.

Shaulian, E., and Karin, M. (2002). Nat. Cell Biol. *4*, E131–136.

Shaulian, E., Schreiber, M., Piu, F., Beeche, M., Wagner, E.F., and Karin, M. (2000). Cell *103*, 897–907.

Smeal, T., Binétruy, B., Mercola, D., Birrer, M., and Karin, M. (1991). Nature *354*, 494–496.

Vogt, P.K. (2001). Oncogene *20*, 2365–2377.