establishing inflammation to promote earlier steps in cancer progression of skin previously "initiated" by the HPV16 transgenes. In contrast to the results of Daniel et al., $CD4^+$ cells were not required for this early tumor promotion. The discrepancy may relate to effects of different cells of the adaptive immune system at different stages of tumor development and progression. For Daniel et al., deficiency of CD4 cells correlated with a trend toward earlier lesions (hyperplasia versus dysplasia at 6 months) and a small but significant difference in incidence of squamous cell tumors at a later stage of tumor progression (at 12 months).

The observations of de Visser et al. raise a number of questions:

• Are antibodies mediating this tumor promotion, or might B cell-derived chemokines, cytokines, or other soluble molecules?

• If the active principle is antibody, what is the specificity? Cutaneous bacterial symbionts or pathogens, autoantigens (including the HPV transgene products), or products of mutations in transformed cells? Tantalizingly, de Visser et al. briefly relate unpublished observations that early development of tumors is not different in germ-free mice, suggesting that bacteria are not driving antibodies.

• If antibodies, what are the mechanisms for eliciting inflammation (Figure 1)? Signaling through activating Fc receptors on myeloid cells to initiate production of inflammatory molecules? Complement activation producing the anaphylatoxins C3a and C3b to drive inflammation through complement receptors on myeloid cells? Both?

• Do T cells play a role in the observations? Importantly, the authors could not rule out the complete absence of T cells in their adoptive transfer experiments.

If antibodies are found to be the mediators of the inflammatory response early on during carcinogenesis in this model, the answers to these questions will become relevant to developing strategies for prevention of cutaneous epithelial tumors and possibly other cancers.

Alan N. Houghton,[1,*] Hiroshi Uchi,[1] and Jedd D. Wolchok[1]

[1]Memorial Sloan-Kettering Cancer Center, New York, New York 10021
*E-mail: a-houghton@ski.mskcc.org

Selected reading

Balkwill, F., Charles, K.A., and Mantovani, A. (2005). Cancer Cell *7*, 211–217.

Berenblum, I., and Haran, N. (1955). Br. J. Cancer *9*, 268–271.

Daniel, D., Meyer-Morse, N., Bergsland, E.K., Dehne, K., Coussens, L.M., and Hanahan, D. (2003). J. Exp. Med. *197*, 1017–1028.

de Visser, K.E., Korets, L.V., and Coussens, L.M. (2005). Cancer Cell, this issue.

Dunn, G.P., Bruce, A.T., Ikeda, H., Old, L.J., and Schreiber, R.D. (2002). Nat. Immunol. *3*, 991–998.

Foulds, L. (1954). Cancer Res. *14*, 327–339.

Nathan, C., and Sporn, M. (1991). J. Cell Biol. *113*, 981–986.

Shope, R.E., and Hurst, E.W. (1933). J. Exp. Med. *58*, 607–624.

Siegel, C.T., Schreiber, K., Meredith, S.C., Beck-Engeser, G.B., Lancki, D.W., Lazarski, C.A., Fu, Y.X., Rowley, D.A., and Schreiber, H. (2000). J. Exp. Med. *191*, 1945–1956.

DOI: 10.1016/j.ccr.2005.04.026

Chromosomal instability in mouse metastatic pancreatic cancer—it's Kras and Tp53 after all

A human pancreatic cancer progression model from intraepithelial neoplasia to ductal adenocarcinoma has been proposed. This process has been modeled in the mouse by activation of mutant *Kras* in pancreatic progenitor cells. In this issue of *Cancer Cell*, Hingorani et al. (2005) present a modification of their initial model by introducing a mutant Tp53. This combination of genetic alterations leads to rapid and increased frequency of neoplasia progression resulting in pancreatic cancers that manifest chromosomal instability in the presence of apparent intact telomeres. These findings introduce *Tp53*-mediated chromosomal instability as key event for carcinoma development in this mouse model.

Pancreatic cancer likely reflects a model type of cancer displaying essentially all molecular and biological cancer hallmarks, such as genetic and epigenetic alterations, chromosomal instability, progression from preneoplastic lesions to an invasive and metastatic phenotype, and virtually complete resistance to any therapeutics tested so far. Along with this depressing clinical situation goes a considerable insight into the genetic and cellular events from the earliest preneoplastic lesions, found in as many as 50 percent of normal pancreata in the elderly population (Hruban et al., 2004), to late metastatic disease (Figures 1A and 1B). Due to significant progress in the development of genetically engineered murine models of human cancers (Van Dyke and Jacks, 2002), mouse models of pancreatic cancer have evolved that mimic the human disease genetically and morphologically in an astonishing way. By conditional activation of endogenously expressed oncogenic $Kras^{G12D}$ in the pancreas of mice, Hingorani, Tuveson, and colleagues were able to induce preneoplastic lesions that eventually progress to invasive and metastatic pancreatic adenocarcinoma (Hingorani et al., 2003 and Figure 1C). However, invasive and metastatic cancer developed at a considerable advanced age in mice, revealing a rather slow progression considering that PanIN-3 lesions can already be detected at the age of 4–6 months to the time of full-blown pancreatic cancer at around 12–15 months of age. By introducing tissue-specific deficiency of the Ink4a/Arf tumor suppressors, often mutated or silenced in

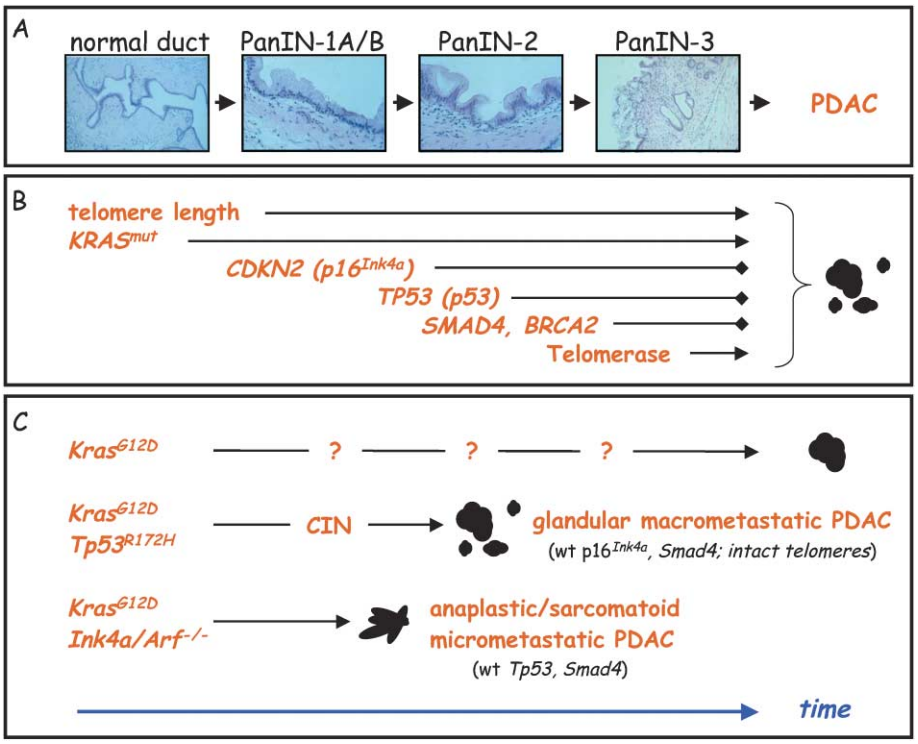

Figure 1. Progression model of pancreatic cancer

A: Histology of pancreatic intraepithelial neoplasia (PanIN) in specimens of human pancreas, which progress to pancreatic ductal adenocarcinoma (PDAC).

B: Genetic alterations in microdissected human PanIN lesions summarized to a genetic progression model.

C: Modeling pancreatic cancer in the mouse ($Kras^{G12D}$, Hingorani et al., 2003; $Kras^{G12D}$;$Tp53^{R172H}$, Hingorani et al., 2005, $Kras^{G12D}$;Ink4a/$Arf^{-/-}$, Aguirre et al., 2003).

preneoplastic human lesions and believed to be necessary for progression of PanIN lesions, into this model, DePinho and colleagues noted an accelerated progression and lethal invasive tumor burden at around three months of age (Aguirre et al., 2003 and Figure 1C). Interestingly, however, no mutations in other genes often altered in human pancreatic cancer, and no macroscopic metastases into other organs typically invaded in human pancreatic cancer, were detectable. In addition, these tumors, despite their high local aggressiveness, showed a rather benign cytogenetic profile. Thus, despite the tremendous advances in mimicking the human disease from early transgenic to these highly elaborated models, some of the key events in pancreatic carcinogenesis have not evolved so far. Besides the development of metastatic tumor phenotypes during the carcinogenic process, chromosomal instability, another hallmark of human cancers, has been very hard to recapitulate in the mouse. In fact, although human epithelial carcinomas, and especially pancreatic cancers, display a high degree of genomic instability, murine models of all kinds of spontaneous cancers have shown a surprisingly bland cytogenetic profile. Since chromosomal instability has been associated with telomere dysfunction, and because telomeres of mice are much longer than human ones, differences in telomere function and dynamics have been considered to be one of the reasons why spontaneous carcinomas are relatively rare in mice, which more often develop lymphomas and sarcomas (Sharpless and DePinho, 2004). Accordingly, mice with telomeric dysfunction in a $p53^{+/-}$ background indeed show a shift in tumor spectrum toward epithelial cancers (Artandi et al., 2000). However, this view may be challenged by a new report published in this issue of *Cancer Cell*.

By introducing a point mutation in the *Tp53* tumor suppressor gene (*Tp53R172H*) into the aforementioned $Kras^{G12D}$ mouse, Hingorani et al. (2005) now seem to have taken a further step in recapitulating human pancreatic cancer. In this model, a structural mutant $Tp53^{R172H}$ is activated the same way as oncogenic $Kras^{G12D}$, leading to targeted expression in pancreatic cells by using PDX-1-Cre mice. Interestingly, concomitant expression of endogenously controlled $Kras^{G12D}$ and $Tp53^{R172H}$ led to an invariant loss of the wild-type *Tp53* allele and halved the time of progression from preneoplastic PanIN lesions to full-blown metastatic pancreatic cancer. In contrast to the highly invasive local tumors seen in mice with concomitant $Kras^{G12D}$ expression and biallelic deficiency of *Ink4a*/*Arf*, these mice developed macroscopic metastases to organs also affected in the human disease. Somewhat surprisingly, however, given the usually observable lack of large-scale genetic alterations in murine epithelial malignancy models, Hingorani et al. (2005) also found induction of widespread chromosomal instability with aberrant chromosomal rearrangements and nonreciprocal translocations. Moreover, these alterations occurred in the setting of apparently intact telomeres (Figure 1C).

An increasing amount of data points to an important role of chromosomal instability and aneuploidy in epithelial carcinogenesis, possibly enabling cancer cells to accumulate mutations needed for as different tasks as stromal interaction, angiogenesis, extravasation, and metastasis. While it is not clear in what exact way chromosomal instability may be essential for tumorigenesis, several studies have reported its occurrence early in tumor development and even in preneoplastic lesions. How then might chromosomal instability have arisen in the mouse model presented by Hingorani et al.? In contrast to the aforementioned $Kras^{G12D}$;*Ink4a*/*Arf* deficiency model, in which biallelic loss of *Ink4a*/*Arf* under continuous oncogenic $Kras^{G12D}$ stimulus led to aggressive locally invasive tumors, expression of $Tp53^{R172H}$ results in loss of the wild-type *Tp53* allele, possibly as a consequence of high selection pressure against *Tp53* after $Kras^{G12D}$-induced cellular stress. Although the question which of these, the initial alteration in Tp53 function or the later loss of the wild-type allele, lead to chromosomal instability has not been formally addressed, mutation in *Tp53* with subsequent acquisition of oncogenic properties may likely be the driving

force for chromosomal instability and additional genetic alterations, including LOH of wild-type *Tp53*, eventually leading to the observable highly metastatic phenotype. In this scenario, chromosomal instability might develop at a rather early time point, as is suggested by Hingorani et al., possibly in PanIN-3 lesions. As the appearance of chromosomal instability is probably of diagnostic and prognostic relevance, this question certainly needs further investigation. What, if any, role telomere function has in this setting is far from being clear. Interestingly, telomere dysfunction in this model may not be required due to the ability of mutated *Tp53* together with oncogenic $Kras^{G12D}$ to induce large-scale genomic instability, a hypothesis challenging our understanding of the development of epithelial cancers in man and mice. Indeed, mice with targeted insertion of structural or contact mutations in the endogenous *Tp53* gene reveal an altered tumor spectrum with an increased incidence of total malignancies and notably of epithelial cancers (Olive et al., 2004). Thus, missense mutations in the *Tp53* gene seem to increase the oncogenic potential of cells, also acknowledged by the fact that *Tp53* is most often not deleted, but rather altered, by missense mutation followed by LOH in malignancy. Therefore, given this new data from mice with endogenously expressed mutated *Tp53*, it may be fair to say that simply knocking out *Tp53* as done in multiple studies in the past has been an oversimplification of the complex *Tp53* network. Compatible with this hypothesis are data from the *Elastase-TGFalpha*;$Tp53^{+/-}$ mouse, in which a rather characteristic pattern of genetic alterations, but not this degree of chromosomal instability, was notable (Wagner et al., 2001; Schreiner et al., 2003). In the future, it will be of great importance to identify pathways interacting with mutant *Tp53* as well as to find therapeutic approaches for inhibiting its oncogenic function. As missense mutations in *Tp53* seem to make the difference regarding the development of genetic instability and evolution of epithelial cancers, it is interesting to note that the selection pressure for Tp53 mutations in mice does not seem to be as high as in humans, an issue which should enforce further investigations.

Interestingly, in PDX-1-Cre mice, activation of mutated $Kras^{G12D}$ and $Tp53^{R172H}$ already occurs during embryogenesis. However, and somewhat surprisingly, pancreatic tumorigenesis seems to develop only in the adult organ, with the very first PanIN-1 lesions occurring not much earlier than in $Kras^{G12D}$ mice without *Tp53* mutation. Could this mean that control of cell division in the embryonic pancreas is more tightly controlled than in the adult pancreas, thus preventing aberrant DNA replication and conferring robust genomic stability? Although not well studied, evolutionary pressure has possibly led to redundant systems preventing the occurrence of genomic instability during embryogenesis.

Some questions remain unanswered. Does the metastatic phenotype as seen in this mouse represent a uniform genetic pathway occurring in this specific genetic setting, or do the metastases represent clonal events with a variety of genetic events generating a similar phenotype? What genes and signaling pathways are affected? What effect may other mutated forms of *Tp53* elicit in this model, such as the contact mutation $Tp53^{R270H}$? Might we see different biological behaviors by altering missense mutations within the *Tp53* gene? Are the observable tumors and metastases dependent on mutated Tp53 function, and most importantly, is genetic instability and the early development of metastatic pancreatic cancer reversible by knocking down mutated Tp53? Is there a therapeutic window?

In summary, the introduction of conditional point-mutated *Tp53* has led to the recapitulation of important and previously undescribed features of human pancreatic cancer in the mouse. Chromosomal instability can now be appreciated as a key event in the development of metastatic carcinomas in the mouse, adding an important new piece to the puzzle of this nearly untreatable tumor.

Jens T. Siveke[1] and
Roland M. Schmid[1,*]

[1]2nd Department of Internal Medicine,
Technical University of Munich, Klinikum
rechts der Isar, Ismaningerstrasse 22,
81675 Munich, Germany
*E-mail: roland.schmid@lrz.tum.de

Selected reading

Aguirre, A.J., Bardeesy, N., Sinha, M., Lopez, L., Tuveson, D.A., Horner, J., Redston, M.S., and DePinho, R.A. (2003). Genes Dev. *17*, 3112–3126.

Artandi, S.E., Chang, S., Lee, S.L., Alson, S., Gottlieb, G.J., Chin, L., and DePinho, R.A. (2000). Nature *406*, 641–645.

Hingorani, S.R., Petricoin, E.F., Maitra, A., Rajapakse, V., King, C., Jacobetz, M.A., Ross, S., Conrads, T.P., Veenstra, T.D., Hitt, B.A., et al. (2003). Cancer Cell *4*, 437–450.

Hingorani, S.R., Wang, L., Multani, A.S., Combs, C., Deramaudt, T.B., Hruban, R.H., Rustgi, A.K., Chang, S., and Tuveson, D.A. (2005) Cancer Cell, this issue.

Hruban, R.H., Takaori, K., Klimstra, D.S., Adsay, N.V., Albores-Saavedra, J., Biankin, A.V., Biankin, S.A., Compton, C., Fukushima, N., Furukawa, T., et al. (2004). Am. J. Surg. Pathol. *28*, 977–987.

Olive, K.P., Tuveson, D.A., Ruhe, Z.C., Yin, B., Willis, N.A., Bronson, R.T., Crowley, D., and Jacks, T. (2004). Cell *119*, 847–860.

Sharpless, N.E., and DePinho, R.A. (2004). J. Clin. Invest. *113*, 160–168.

Schreiner, B., Baur, D.M., Fingerle, A.A., Zechner, U., Greten, F.R., Adler, G., Sipos, B., Kloppel, G., Hameister, H., and Schmid, R.M. (2003). Genes Chromosomes Cancer *38*, 240–248.

Van Dyke, T., and Jacks, T. (2002). Cell *108*, 135–144.

Wagner, M., Greten, F.R., Weber, C.K., Koschnick, S., Mattfeldt, T., Deppert, W., Kern, H., Adler, G., and Schmid, R.M. (2001). Genes Dev. *15*, 286–293.

DOI: 10.1016/j.ccr.2005.04.025