induced tumor formation in gene targeted or transgenic mice might provide insights relevant to human carcinogenesis. An instructive and relevant parallel to the current findings was the initial excitement generated by several papers that described FasL expression on tumors. FasL, a member of the TNF family of pro-apoptotic molecules, can induce the death of cells that express the cognate death ligand Fas. Theoretically, this could lead to T cell deletion and "immune privilege" status for the tumor. The resulting predictions that FasL overexpression on tissue allografts would delay or prevent rejection were not fulfilled. In fact, implantation of FasL-overexpressing cells in mice resulted in precisely the opposite result: even more rapid rejection and abscess formation in the transplanted tissue (reviewed in Restifo, 2000).

Along with the present report of Dong and collaborators, the literature currently contains several others postulating or purporting to show inhibition of T cell effector function as a result of interaction with cancer cells (Figure 2). These reports include models where cancer cells may (i) overexpress serpins (protease inhibitors) that block granzyme B-mediated apoptosis (Medema et al., 2001); (ii) downregulate expression of purported granzyme receptors such as the 280 kDa mannose-6-phosphate receptor (Motyka et al., 2001), also blocking apoptosis; or (iii) transmit a signal to cytotoxic T lymphocytes that blocks the PI-3 kinase pathway, leading to failure of perforin and granzyme secretion due to dysregulated granule trafficking (Radoja et al., 2001). Each of these hypotheses requires further testing in appropriate animal models, and extreme caution is essential before applying the apparently logical consequences of these "lessons" to human cancer therapy.

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salvador—The persistence of proliferation

Despite years of extensive studies on genes that regulate proliferation and cell death, two processes that must be tightly coordinated throughout development to regulate cell number, remarkably few genes have been shown to affect both processes. Using an elegant genetic screen in the fly eye, Tapon et al. (2002) have identified a gene, *salvador*, which is especially significant, because it not only regulates and coordinates both exit from the cell cycle and apoptosis, but also has a human homolog that may play a key role in tumorigenesis.

The fly eye develops from merely 30 progenitor cells into an exquisitely precise and highly ordered structure consisting of approximately 800 individual units or ommatidia and numbering more than 15,000 cells. To reach this final form, which is so beautifully regular that it has been called a "neurocrystalline lattice" (Ready et al., 1976), the signals for proliferation, patterning, exit from the cell cycle, differentiation, and cell death all must be carefully regulated and coordinated. The fly eye has provided a sensitive system for the discovery of genes and regulatory networks that control these processes, but noticeably, few genes have been shown to regulate both exit from the cell cycle and execution of the apoptotic program—two developmental events that must be tightly coordinated to regulate cell number. When these processes are uncoupled or disrupted, the host is at risk for developing a tumor.

Tumor suppressors are genes that, when inactivated, confer a proliferative advantage over normal cells. This can occur by a variety of different mechanisms and can lead to the development of tumors, disorganized masses of tissue that can cause the death of the host—whether that host is a fruit fly (Woodhouse et al., 1998) or a human being. In fact, many, if not all, human cancers involve the inactivation of tumor suppressors (Hanahan and Weinberg, 2000). These genes comprise an important and diverse group, and their discovery and characterization have helped us develop a deeper understanding of cancer cell biology. To date, a large number of tumor suppressor genes have been reported in flies, and many of them have human homologs that

have been implicated in tumorigenesis (Huang et al., 1999; Potter et al., 2001; Tapon et al., 2001).

In 1995, Xu et al. described a powerful screen to use the then recently developed technique of mitotic recombination using Flp-recombinase in the fly eye as a means to discover new tumor suppressor genes (Xu et al., 1995). By inducing mitotic clones early in eye development and comparing the size of the resulting mutant clones with their wild-type counterparts, the researchers were able to isolate mutations which conferred a growth advantage to the mutant tissue and even caused cancer-like masses to appear in the fly. The authors of a recent paper in *Cell* (Tapon et al., 2002) conducted a similar screen that has identified mutations in several tumor suppressors and negative regulators of proliferation. They now report the characterization of an interesting gene they have named *salvador* (*sav*) after the surrealist painter Salvador Dalí, who once claimed (before his death) to be immortal.

In normal retinal development, progenitor cells proliferate early and are later patterned and recruited sequentially to adopt the various cell fates and form ommatidia, the individual light-sensing structures of the compound eye that are made of photoreceptors and supporting cells. The last cells recruited into the final structure become interommatidial cells, cells that normally form a single layer between ommatidia, leaving an excess of approximately 2000 cells which are then pruned during a period of apoptosis. This cell death results in a tight lattice of interommatidial cells. Cells mutant for *sav* are at a proliferative advantage over their wild-type counterparts. When a mitotic clone is induced early in eye development to generate a *sav* mutant cell and a wild-type cell, the mutant one eventually contributes more cells to the fully developed eye. Upon sectioning the eye, one sees that while ommatidial patterning is generally unaffected, the mutant tissue contains many more interommatidial cells, suggestive of a defect in cell death. In clones of the most severe *sav* allele, sav^3, the mutant tissue protrudes out in folds. In addition, when sav^3 clones are induced in other parts of the body, mutant tissue develops into tumorous growths.

How does loss of *sav* lead to such a phenotype? First, BrdU labeling experiments in the developing eye demonstrate that mutant cells continue to proliferate for 12 to 24 hr after their wild-type counterparts have exited the cell cycle and begun to differentiate. The mutant cells have elevated levels of CyclinE RNA and protein, a condition that prevents exit from the cell cycle. Thus, one function of *sav* is to reduce CyclinE levels, at least in part by regulating its transcription. Eventually, however, the mutant cells do stop dividing, presumably because other mechanisms are at work to inhibit CyclinE.

The next defect that is apparent in *sav* mutant tissue is a dramatic reduction in cell death in the interommatidial cells; at a point when there is widespread cell death in wild-type tissue, mutant cells do not die. Protein levels (but not RNA levels) of a key regulator of cell death, DIAP1 (*Drosophila* inhibitor of apoptosis 1), are elevated, suggesting that increased levels of DIAP1 in *sav* clones, caused by a transcriptionally independent mechanism, may overcome proapoptotic signals. Indeed, overexpression of Reaper or Hid, two potent proapoptotic proteins that normally inhibit DIAP1 by binding to it and targeting it for degradation (Martin, 2002), fails to have an effect in tissue mutant for *sav*. Notably, an especially potent form of Hid that is resistant to inactivation by phosphorylation, Hid-Ala5, can overcome the elevated DIAP1 levels and still induce some cell death in *sav* clones. This indicates that *sav* is not absolutely required for apoptosis to occur and that *sav* may normally function to downregulate basal DIAP1 levels posttranscriptionally.

The Sav protein is 608 amino acids long with two putative WW domains and a C-terminal coiled-coil region. It is expressed in the developing eye in regions where cells are arrested in the cell cycle. *salvador* is also expressed in other tissues, for mutant *sav* clones in the notum and the haltere also cause tumors. Interestingly, the region of Sav containing the WW domains can bind in vitro to a serine-threonine kinase called Warts/LATS, another tumor suppressor whose phenotype overlaps substantially with that of *sav* but is somewhat more severe (Justice et al., 1995; Xu et al., 1995). Mutant clones of *warts* also develop into tumors, contain extra interommatidial cells, display increased levels of CyclinE, and show almost no apoptosis. (Significantly, mice lacking a homolog of Warts, LATS1, develop hyperplasia and tumors in several tissues [St John et al., 1999]. Additionally, a recent report indicates that human LATS1 regulates both the cell cycle and cell death [Xia et al., 2002].) Warts and Sav may work together synergistically, since overexpression of either Sav or Warts in the fly eye causes very subtle or no defects, yet simultaneous overexpression of both induces broad cell death and causes the eye to be considerably smaller. However, despite this interaction and the finding that the proteins can associate in vitro, the two genes are not in a linear pathway; the double mutant phenotype is more severe than either single mutant phenotype. Thus, questions remain about the relationship of Sav and Warts.

sav is widely conserved with homologs in worms and mammals. Also, the human *sav* ortholog, hWW45, maps to a genomic region subject to allelic loss in several different cancers in a variety of tissues (Valverde, 2000). Because of this, the authors tested whether hWW45 is mutated in cancer cell lines. After analyzing cell lines derived from multiple tissues, they found that two renal cell carcinoma cell lines, ACHN and 786-O, both have homozygous deletions in hWW45. Even more suggestive, these deletions overlap in only a 21 kB region, a segment that apparently contains only exons 3–5 of hWW45 and no other transcriptional units. Since hWW45 is ubiquitously expressed in adult tissues (Valverde, 2000), perhaps further studies will reveal that the gene is involved in other cancers as well.

As any interesting study does, this paper raises some questions. First, an important question about the *sav* mutant phenotype remains unanswered. The authors elegantly demonstrate a role for *sav* in regulating both cell cycle exit and apoptosis, two findings that explain one aspect of the mutant phenotype: the excess of interommatidial cells. However, there is another important aspect to the phenotype of *sav* mutant clones. These clones have considerably more ommatidia than their wild-type counterparts, indicating that the loss of *sav* provides mutant cells with a proliferative advantage. Do *sav* mutant clones have a shorter or quicker cell cycle, or do they just proceed through an extra division or two? The authors did not detect a gross difference in cell cycle parameters, but they point out that even a very subtle difference undetectable by their assays would be amplified over several cell cycles to cause an obvious size discrepancy between mutant and wild-type clones.

How does *sav* confer such a growth advantage?

Furthermore, several questions persist about *sav*'s mechanism of action. First, although *sav* clearly affects transcriptional levels of CyclinE, how is the signal transduced to the nucleus and integrated with other transcriptional regulators of CyclinE? Also, how exactly does Sav keep DIAP1 levels down in a transcriptionally independent way? Finding in vivo binding partners of Sav will probably help answer these questions.

Knowledge of such binding partners may shed light on some other important questions and help fit *sav* into a pathway (or several pathways). For example, is *sav* in fact part of the Reaper/Hid pathway, or is it involved in a parallel pathway? Might *sav* even play a direct role in helping Hid and Reaper target DIAP1 for degradation? Also, what is the relationship between *sav* and the MAPK pathway, a group of genes that also regulates both the cell cycle and apoptosis?

Finally, the *sav* study raises several cancer-related questions. For instance, can the transformed phenotype of the hWW45 null cell lines be attributed to the lack of that gene? Can *sav*'s role in tumorigenesis in the mammal be demonstrated in vivo; i.e., will a tissue-specific knockout of the mouse *sav*/hWW45 homolog, mWW45, induce the development of tumors in that tissue? If so, will they be capable of metastatic growth, or will the cells behave as in the fly, terminally differentiating and remaining in situ? Ultimately, we would like to know if mutations in hWW45 cause tumors in humans.

Clearly, the discovery of *sav* is an exciting one. Hopefully, *salvador* can paint us a clearer picture of tumorigenesis.

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