2 p27, A Prognostic Indicator Reflecting …?

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Contents

1. SUMMARY

Over the last five years, the expression of p27kip1, a cyclin-dependent kinase inhibitor, has proved to be a strong prognostic indicator for long-term survival of patients with tumors of the colon, breast, prostate, lung, pituitary, and many other tissues. Tumors arising in these organs that were not expressing p27 protein tended to be more aggressive and patients had a poorer clinical outcome. However, it is not clear why p27 was such a strong prognostic indicator in multiple tissues. Furthermore, before p27 is brought into widespread clinical use, prospective studies will be required to validate, in advance, a clinical course or response to therapy. Without the essential knowledge of what low p27 prognosticates, vis a vis the evolution of the tumor, validation will be difficult. Because there is no possibility of determining directly how low p27 expression facilitates tumor development in humans, we have turned to developing mouse models.

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However, we had to first ask the following questions: Does p27 deficiency contribute to tumor development in the mouse? If p27 deficiency contributed to tumor development, does it mimic the human condition, i.e., were tumors more aggressive? Then, if they were more aggressive, what was the mechanism underlying this? Before we begin to discuss these issues, I apologize to the many investigators whose work will be either uncited or cited only by review.

2. CHANGES IN p27 ACTIVITY/ABUNDANCE ARE PROGNOSTIC IN MANY TUMORS

The diagnosis of tumor stage and grade is quite subjective and largely depends on the experience of the pathologist with that specific lesion. Even among experienced pathologists, there are disagreements about clincial stage and grade. The intermediate tumors, those that are clearly more advanced than just hyperplasia but not yet obviously aggressive, present a substantial problem. Because treatment is based on the severity of the disease and the likelihood of its progression to a more severe disease, it is important to remove the ambiguity surrounding diagnosis and prognosis. Well-developed and well-understood molecular markers of disease stage and prognosis will succeed in this endeavor.

Over the last five years the expression of p27kip1, as determined by immunohistochemistry, proved to be a strong prognostic indicator of patient survival of tumors of the colon, breast, prostate, lung, pituitary, bladder, and glioma (reviewed by *1–4*). Generally, tumors where low p27 expression was prognostic for severity were of intermediate grade. However, there were exceptions. For example, in Burkitt's lymphoma, increased p27 was associated with increased aggressiveness *(5,6)*. Furthermore, cytosolic localization was observed in ovarian cancers of low malignant potential *(7)* and Barrett's associated adenocarcinoma *(8)*. While these differences are organ/tumor type specific, they probably underlie the complexity of the regulation of p27 abundance (Fig. 1).

Stabilization of p27 in Burkitt's lymphoma was associated with an increase in cyclin D3 *(5,6)*, which is consistent with the inactivation of p27 by sequestration as originally proposed *(9,10)* and the observation that myc induced accumulation of D-type cyclins leads to p27 sequestration *(11)*. However, the mechanism by which cyclin D3-cdk association stabilizes p27 remains to be determined—does it block the phosphorylation of p27 on T187 preventing ubiquitin-dependent protein degradation, or does it block the interaction of p27 with the skp2-containing E3?

Cytosolic localization of p27 was observed in BAA *(8)* and ovarian tumors of low malignant potential (LMP) *(7)*, as well as in a diverse number of sarcomas (P. Capodieci, C. Cordon-Cardo, AK, unpublished data), and even in a 3T3 cell line *(12)*. However, the molecular mechanism remains a mystery. Candidate proteins that might affect p27 localization and that could be mutated in human tumors abound. These include jab1 *(13)*, Nup50 *(14)*, and TSC2 *(15)*.

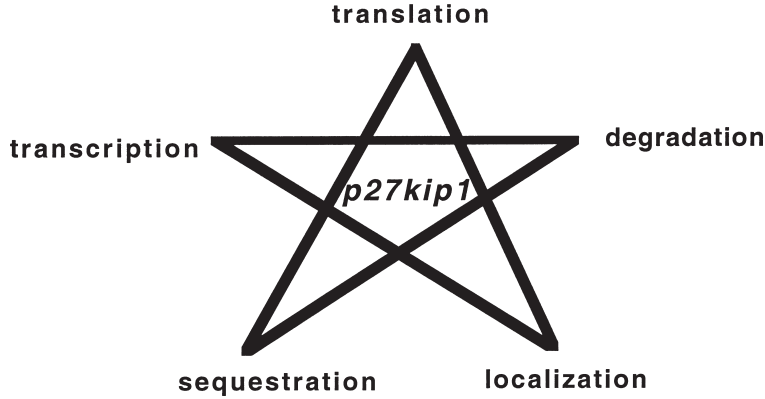

Fig. 1. Processes that regulate the activity and/or abundance of p27kip1. Current investigations are focused on the elucidation of the molecules involved in each of the processes, and the biologic significance of any one of them has not been determined.

The mechanisms leading to the lack of p27 staining are unclear. The absence of p27 protein has rarely, if ever, been attributed to loss of the chromosomal location of the gene (12p13; *16–18*), nor has the absence of protein been well-correlated with loss of mRNA *(1–4)*. However, recent evidence from studies on carcinogen-induced tumor development in p27+/– mice *(19,20)*, and in tumor progression in p27+/– mice intercrossed with Rb+/– mice *(21)* indicated that gene dosage was an important factor. Thus, unlike many genes that require LOH to be implicated as encoding tumor suppressors, p27 is haploinsufficient, and the conclusion that mRNA does not change may need to be re-evaluated keeping in mind that a 50% reduction may not have been readily determined by the most widely used techniques.

On the other hand, it is generally accepted that the inability to detect p27 is due to post-transcriptional changes in protein abundance, and in some cases, it has been suggested to be due to increased ubiquitin-dependent proteolysis *(22–25)*. The strength of this conviction is apparent in the sense that reviews often offer no other alternative *(1–4)*. However, the data supporting this conclusion was derived from experiments generating protein extracts from tumor samples and measuring p27 ubiquitination *(23,24)* or the loss of protein *(25,26)*. This only allows a comparison of proteolytic activity in tumors to that seen in normal tissues. Because p27 degradation is associated with commitment to the cell cycle and entry into S-phase, wouldn't tumor extracts have an increased amount of activity? To overcome this concern, there are a number of studies showing that p27 expression in a tumor was not correlated with proliferation as measured by either Ki67 or MIB reactivity (for example *see* refs. *27–29*) and that these markers together may be more informative than either alone. This reduced the possibility

that the prognostic significance of p27 would be associated solely with proliferation, but it does not indicate that proteolysis is the reason for low p27 expression.

Tumors arise as a consequence of cells inappropriately executing the decision to proliferate or withdraw from the cell cycle, they are not simply a decision to continue through the cell cycle. The regulation of p27 abundance between cycling and noncycling states is at the level of translation *(30,31).* As most cells in a tumor are not proliferating, at least as judged by Ki67 or MIB staining, they are not in the cell cycle, but rather may be in the transition of G_0-to-G_1. A molecular understanding of the mechanisms regulating translational control of p27 mRNA is only now being elucidated (*32*; A. Vidal, S. Millard, AK, unpublished data).

Perhaps when our understanding of the molecular mechanisms regulating p27 abundance is complete, or better defined than the current synthesis, degradation, and location, we would understand what the loss of p27 represents. Nevertheless, even if we do not know the mechanism that accounts for the loss of p27, a reduction in the amount of functional nuclear p27 protein is prognostic.

3. TUMORS ARE THE SUM OF PROLIFERATION AND OTHER CHANGES IN THE CELL

Recently, reviews on the changes that occur during progression from normal cell to tumor mass were scribed by Hanahan and Weinberg *(33).* There is very little to add to this, however, it is important for this discussion to review some of the landmarks of tumor development. First, cells must be proliferating. Second, the proliferating cell must not be undergoing apoptosis. Third, the proliferating and living cell must suspend or bypass mortality controls. Of course, even given all these changes, a tumor does not develop in a homogenous environment like a tissue-culture dish, rather it develops in an organism and is affected by its interactions with other cells and on environmental factors. Thus, tumors must induce angiogenesis, and tumor cells often alter their interaction with neighboring cells, alleviating the ability of normal cells to maintain the tissue in a clear, but as of yet molecularly undefined, homeostatic state. Finally, tumor cells that have migrated to distant site must also evolve mechanisms of coping in these strange and often hostile environments.

Not all tumors have the need for angiogenesis or develop metastatic potential; however, all undergo changes in proliferation, apoptosis, and senescence. As we are focusing on p27 and the role that it might play in tumor development, it is helpful to consider how cells move from quiescence into the cycle, and back again.

The effect of mitogenic and anti-mitogenic signals on progression through G_1 phase, and the choice between either commitment to the cell cycle and eventual DNA replication or withdrawal from the cell cycle and perhaps acquisition of a differentiated phenotype, is made by controlling the activation status of the

cyclin-dependent kinases. Entry into S-phase requires the activation of two cyclin-dependent kinases, cdk4 and cdk2, which participate together in the inactivation of Rb and the induction of E2F-dependent transcription *(34)*. Although the activation of these kinases is necessary for S-phase entry, it is important to note that there is no evidence in primary cultures of mammalian cells to suggest that this is sufficient to account for all the functions of mitogens required for S-phase entry. Some targets of the cyclin-cdk complexes include pocket-proteins, such as Rb; the cdk inhibitors, such as p27 *(35–40)*; and molecularly defined targets within the centrosome *(41)*. The consequence of target phosphorylation is quite varied. Rb phosphorylation alters gene transcription through changes in HDAC-association and E2F1 association *(42)*. p27 phosphorylation alters its stability. The role of phosphorylation in the centrosome is unclear.

Mitogenic signals, often through the RTKs, induce the synthesis of cyclin D1, inhibit the degradation of cyclin D1, and foster the assembly of cyclin D1 with cdk4 *(43–45)*. Additionally, mitogens can suppress the translation of p27 *(30)*. With an increase in steady-state accumulation of cyclin D-cdk4, the availability of p27 to bind to cyclin E-cdk2 is limited, and thus cyclin E-cdk2 activity could begin to accumulate *(10)*. Once cyclin E-cdk2 accumulates it phosphorylates p27 and initiates ubiquitin-dependent degradation of the protein *(35–38,40)*. This appears to be sufficient to allow for an irreversible commitment to cdk activation in the presence of mitogen.

Anti-mitogenic signals can impact the decision to proliferate by directly increasing the amount of Ink4-type inhibitors. For example, transforming growth factor-β (TGF-β) can induce p15 accumulation, p15 complexes with cdk4 preventing the formation of cyclin D-cdk4 complexes. This prevents sequestration of p27 and the amount of p27 will be sufficient to inhibit cyclin E-cdk2 *(46)*. Likewise, antimitogenic signals can directly induce p27 translation by interfering with the rho-dependent mitogenic signal-transduction pathways that suppress it (A. Vidal, S. Millard, AK, unpublished data). In both examples, the anti-mitogenic signal would antagonize the mitogenic signal; however, the final decision would depend on the equilibrium established between cyclin D-cdk4/p27 and cyclin E-cdk2.

There are a number of cdk inhibitors akin to p15 and p27, the Ink4 class (p15, p16, p18, p19) and the Kip class (p21, p27, p57), respectively. These proteins are expressed in a cell-type specific manner, and it is generally assumed that they carry out similar roles in mediating growth arrest. However, if that was true, then one would expect that the individual cki-deficient mice would have quite similar phenotypes, i.e., problems in the differentiation of cells that express that particular cki, but this does not appear to be the case *(47)*. The reasons for this are not clear; however, there is cell-type and signal specificity to their accumulation and function in promoting growth arrest. There may be additional functions in differentiated cells, or as regulators of growth arrest in response to DNA damage or

nucleotide pool perturbation, or in the assembly of cyclin D-cdk complexes *(48,49)*.

4. HOW CHANGES IN CELL CYCLE REGULATORS MIGHT IMPACT TUMOR GROWTH

For a number of years we have known that the length of time that a cell spends between mitosis and DNA replication can affect its response to the signals that ultimately control its proliferative fate. This was first shown in the response of the simple yeast, *Sacchromyces cerevisiae*, to mating pheromones and was instrumental in uncovering cln3 *(50,51)*. Cancer is a disease where the cell has reduced its dependency on mitogenic signals and no longer responds correctly to anti-mitogenic signals. A few examples from an extensive literature of many mammalian cell types serve to illustrate the fact that G_1 duration affects response: the overexpression of specific D-type cyclins can prevent granulocytic *(52)* or muscle *(53)* differentiation. Cyclin overexpression in fibroblasts will accelerate S-phase entry, but will not prevent growth arrest in response to contact inhibition or complete abolish the need for mitogen. Enforced expression of cdk inhibitors, such as p21 can induce muscle *(54)* or myeloid *(55)* differentiation. In some cases there is no differentiative effect, only a proliferative one. For example, expression of p27 in primary rat oligodendrocyte precursor cells leads to growth arrest but not differentiation *(56)*. Thus we can conclude that mutations that affect G_1-duration have a phenotype similar to one that would affect a growth inhibitory signaling pathway. However, these were all in single cell organisms, explanted cells, or cells grown in culture. Is the same true in a multi-cellular organism?

Mouse genetics has made it possible to examine what overexpression of a cyclin, mutation of a cdk, the absence of a cdk inhibitor, or the mutation of proteins that regulate cdk activity (i.e., cyclin D_1, cyclin E, cdc25, or cdc37), do with regard to tumor development, both spontaneous and carcinogen-induced. There are a large number of these reports on a wide variety of tissues *(57–75)*. The phenotypes of the different cdk inhibitors are discussed individually and have been reviewed *(47,76)*. The phenotypes of the cdk4R24C mutation and the cdk4 knock-out are described in the appropriate references *(77,78)*. Overall, however, individual changes in cdk activity had a relatively modest effect. That might be due to the necessity of activating two cdks, cdk4/6 and cdk2, to drive cells into S-phase *(79,80)*, but even that is not entirely clear, as the activation of cyclin E-cdk2 should bypass the need for cdk4/6 expression by the current model (Fig. 2) and the observations made when cyclin E was knocked-in to the cyclin D_1 locus *(81)*.

Another interpretation is that mutation of the cell cycle, specifically with respects to the proliferation of tumor cells, is kept innocuous by the homeostasis provided by an animal. Proliferating, oncogenically activated cells may be

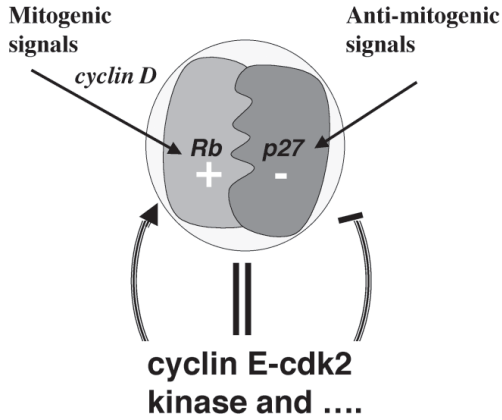

Fig. 2. Cyclin D-cdk complexes and p27 conspire to regulate cyclin E-cdk2. The decision to commit to S-phase correlates well with the activation of cyclin E-cdk2 kinase. Once activated, cyclin E-cdk2 kinase, insures that a strong positive feedback loop is initiated that leads to the increase of cyclin E mRNA, through further inactivation of the Rb-E2F complex, and the elimination of p27kip1, through ubiquitin-dependent proteolysis. Cyclin D-cdk complexes mediate the passage of mitogenic signals to cyclin E-cdk2 through their effects on Rb-E2F complex, and anti-mitogenic signals through their effects on p27.

undergoing apoptosis or senescence continually in the animal. In contrast to the cell cycle changes, when mice were engineered to overexpress or to express mutated forms of many molecules upstream of the cell cycle, i.e., ras, myc, or the her2 receptor, they were obviously tumor prone. Each of these molecules has effects on cell cycle regulators, but also affect proteins that participate in apoptosis and senescence. For example, growth factor cytokines, such as IL-2 *(82)* or c-kit *(83)*, acting through their receptors, often tyrosine kinases, have roles regulating cell proliferation and cell survival, often mediated by interactions through the ras- and PI3-kinase signaling pathways, respectively *(84,85a)*. However, activated ras will induce senescence, presumably through Arf and p21 *(86–88)*. Ras will also intersect the cell cycle: through raf controlling the abundance of the cyclin D-cdk4 complex *(43)*, and through rho controlling the abundance of p27 (A. Vidal, S. Millard, AK, unpublished data). PI3 kinase suppresses apoptosis by activating Akt *(84)*. PI3 kinase activity also can regulate p27 abundance *(85b)*. Additionally, the proliferating cell normally induces a p53 response, which is implicated in apoptosis and/or cell cycle arrest *(86–89)*. The dual nature of p53 rests, at least in part, on its ability to induce p21 *(88,90)* and bax *(91)*, a suppressor of proliferation and an inducer of apoptosis, respectively. Of course, apoptosis and growth arrest can also be p53-independent *(92–95)*.

One way of interpreting this cornucopia of data is that changes in the cell cycle are associated with abnormal proliferation, but are not sufficient to cause abnormal proliferation. Nevertheless, changes in these regulators specifically impact

on the ability of the cell to respond to anti-mitogenic signals. Consistent with this possibility, the tumor phenotypes occurring in these mice expressing myc can be enhanced by overexpression of cyclin D_1 *(63,96)*. Those expressing ras can cooperate with cyclin E *(61)*. Keratinocytes lacking p21 and expressing oncogenic ras form aggressive tumors in nude mice, more so than if they lacked p27 or were wild-type *(97)*. Furthermore, mutation of cdk4 makes cells refractory to the actions of Ink-class inhibitors and the overexpression of cyclins would titrate Kip-class inhibitors. This would also seem to be consistent with the finding that only a few mutants in the cell cycle regulators gave rise to "cancer-like" phenotypes. Specifically those in p27-deficient mice, p18-deficient mice, Ink4a-deficient mice, and E2F1-deficient mice were informative, displaying tumor growth properties. Both E2F1 and p27 (see below) are implicated in regulating the transition between cycling and noncycling cells.

However, a word about the Ink4a locus, as this may be due to a non-cell cycle effect. The Ink4a locus is incredibly complex, It encodes both the cdk4 binding protein, p16, and an alternative reading frame, p19Arf1, which share a second exon and have alternative first exons *(98)*. Deletions often, but not always, remove both reading frames *(99)*. Arf1 interacts with and negatively regulates the p53-mdm2 pathway and myc participates in this process, albeit there is still some disagreement over the exact nature of these interactions *(88,100–104)*. Thus mutation in a single locus would affect both the Rb and p53 pathways and has brought into question what the role of p16 deletion in human tumors really is. At this time, this question is unanswered. There are mutations in the p16 ORF identified in tumors that do not affect the p19 ORF, at least by sequence analysis, suggesting that p16 may be a tumor suppressor. However, in mice, the p19Arf1 deletion fully recapitulates the growth and transformation properties of cells and the tumor development property of mice observed with the p16-/-p19-/- mouse (the original Ink4a deletion) *(105–107)*. The rest of this review will studiously ignore the Arf complexity as this has been described recently *(108,109)*.

Consequently, I would raise the proposal that many of the mutations in cell cycle regulators do not drive cell proliferation, but rather make proliferating cells refractory to the consequence of the signals telling them to stop. The goal then became a direct test of the hypothesis, specifically in relationship to p27, rather than culling the data for the consistent observations.

5. p27 AS A PROGNOSTIC INDICATOR: A CELL REMOVED FROM THE CONSEQUENCE OF ANTI-MITOGENIC SIGNALING

The phenotype of p27 deficient mice was quite striking *(110)*. Our mice expressed an amino truncated protein (Δ51, deleted amino acids 1-51) that failed to bind and inactivate cdks, and two other groups created nullizygous mice *(111,112)*. These lines displayed identical phenotypes. Although p18 deficiency can recapitulate some of the phenotypes below, it appears to be more dependent on strain background *(79,113)*. The mice were larger than their wild-type littermates, had no measurable increase in the serum level of growth hormone (GH), insulin-like growth hormone (IGF-1), or IGF-2, and there was an increase in the S-phase fraction of cells in organs in which proliferation was occurring, such as thymus. However, there were very few discernable developmental defects associated with increased proliferation in p27–/– mice except for deafness *(114,115)* and female infertility *(116)*. These data suggested that p27 was involved in the regulation of cell proliferation in many tissues, but did not directly address how.

Evidence from a number of laboratories suggested that p27 was an input for anti-mitogenic differentiation inducing signals, transducing these to the core cell cycle component, cdk2. This was most clearly demonstrated in our studies of the growth and differentiation properties of oligodendrocyte precursor cells isolated from the brain cortex of neonatal mice *(117–119)*, and the granulosa-to-luteal cell transition following hormone induced ovulation *(116)*. Similar results have been shown in the Organ of Corti and in osteoblasts *(114,115,120)*. In each case, a cell-autonomous increase of p27 protein was correlated with differentiation. Thus in cells where it is expressed, p27 clearly has a role in cell cycle withdrawal induced by differentiation signals. The defects we and others reported in the withdrawal program of p27-deficient cells may have been due to a direct response altering their ability to interpret the anti-mitogenic signals, or an indirect response, i.e., these p27–/– cells may have a shorter G_1 period. On this note, although p27 deficiency does not alter G_1 duration in mouse embryo fibroblasts, it has not been examined in any of the cell types above.

As might be expected from the model that cell cycle mutation alleviates cellular response to anti-mitogenic signals, but does not promote proliferation, p27 deficient mice also developed a number of spontaneous abnormal growths—very low grade tumors. These mice spontaneously develop benign prostatic hyperplasia *(121)*, low-grade C-cell carcinoma of the thyroid *(21)*, and a pituitary intermediate lobe hyperplasia or adenoma *(21,110–112)*. Additionally, carcinogens were able to induce tumor development more efficiently in knock-out and heterozygous mice than in wild-type counterparts *(19,20)*. However, it should be noted that heterozygous mice did not spontaneously develop tumors suggesting that haploinsufficiency at this locus is enough to exacerbate a tumorigenic event, but by itself is not tumorigenic.

Now, in order to test the hypothesis that cell cycle regulators, and p27 specifically, might act to prevent anti-mitogenic response of developing tumor cells, we set out to create a mouse model. Our choice to couple the Rb+/– mouse to a p27–/– background seemed reasonable as much was understood about tumor development in the Rb+/– mouse and the tissues affected were similar to that of the p27–/– mouse. Furthermore, if one had to speculate how an oncogenic event would affect cell proliferation, one needs only examine the linkage between ras and Rb. Thus, we speculated that loss of heterozygosity at the Rb locus would provide the oncogenic event and allow us to determine if p27-deficiency would increase the aggressiveness of the resulting tumors.

Rb–/– mice die between embryonic d 14 and 15, depending on the specific disrupted allele and background of the animals *(122–124)*. Death is associated with apoptosis in neural tissues and a lack of fetal hematopoiesis. Rb+/– mice are viable and lead unremarkable lives early in the postnatal period. However, as the animal ages, there is a remarkable incidence of pituitary adenocarcinoma involving the melanotrophs, and C-cell carcinoma of the thyroid *(125,126)*. Remarkably, this is the same tumor spectrum observed in p27–/– mice. Not surprisingly, both p27 and Rb protein accumulate in the mouse melanotroph *(21,125)*. The highly aggressive pituitary adenocarcinoma is thought to be responsible for death of these animals at approx 10–14 mo of age, depending again on genetic background and the specific mutant allele of Rb.

The natural history of the pituitary adenocarcinoma arising in the Rb+/– animal is quite interesting. All the tumors underwent LOH of the Rb locus. This occurred very early in postnatal development with 94% of the animals having undergone an LOH event by day 90 *(125)*. These Rb–/– cells then re-entered the cell cycle, however those cells innervated by the dopaminergic neuron underwent apoptosis. Dopamine is a potent negative growth regulatory signal for the melanotrophs. At some point during the transition from the early proliferates to tumor, the cells acquire a mutation(s) that allows them to develop into an adenocarcinoma. Because these Rb–/– melanotrophs retained the dopamine receptor, it suggested that the other mutations either prevented innervation or the death due to the "oncogenic activation" of Rb LOH coupled with innervation. These mutations might alter the dopaminergic neuron interaction or the ability of the dopaminergic neuron to signal effectively. Whatever the cause, these tumors acquired resistance to innervation and proliferated uncontrollably, or perhaps even proliferated in a manner that now prevented their innervation.

The aforementioned possibilities suggested that signals that disrupt proliferation-induced apoptosis might alter the latency period of this tumor. As indicated this could occur either by disruption of the negative regulatory signals controlling proliferation, or an inability to activate the apoptosis inducing machinery (Fig. 3). Three crosses of Rb+/– mice to other genotypes led to an exaggeration

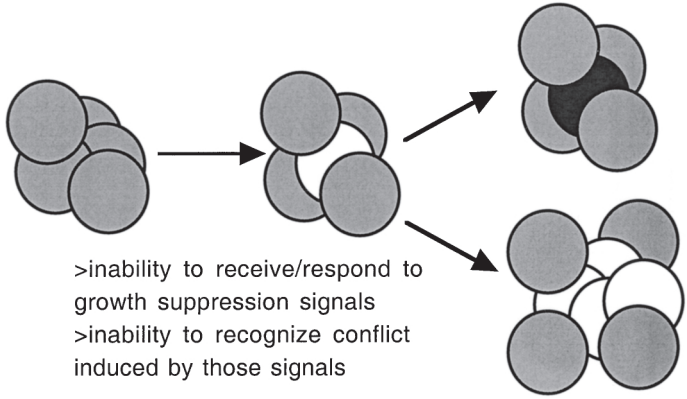

Fig. 3. Possible ways to overcome oncogene induced apoptosis. Following an oncogenic event and the induction of cell proliferation, the cell will either die or senesce before causing any significant tumor to form (top). However, if the cell mutates such that it cannot recognize negative growth regulatory signals from surrounding cells, or cannot initiate an apoptotic pathway perhaps initiated by these conflicting signals, it will continue to proliferate and eventually form a tumor mass.

of tumor phenotype as measured by the classical criteria of a shorter latency period. These included the crossing onto a p53-deficient background *(127)*, a p21-deficient background *(128)*, or a p27-deficient background *(21)*. Although p53 mutation was observed in the original Rb+/– model, the data is consistent with a model wherein the dopaminergic neuron induced death is occurring because of oncogene induced apoptosis, where loss of Rb is the oncogenic event. Likewise, the ability of p21 deficiency to accelerate the tumor may be similar. But what of p27 deficiency?

One possibility is that the loss of p27 prevents the dopaminergic neuron signal from being strong enough to induce p53-dependent apoptosis. In this model, one would have to assume that the cell simply does not recognize the conflict that leads to oncogene-induced apoptosis. However, there is another alternative. The loss of p27 may allow the rate of cell proliferation in the tumor to exceed the rate of apoptosis induced by the dopaminergic neuron. In this scenario, the cells would more rapidly escape the apoptosis-inducing effects of proximity to the neuron. Only now that this model exists can we examine these mechanisms for the aggressiveness associated with low-p27. However, other models are on the horizon. These involve p27 intercrosses to other tumor suppressors such as Pten and inhibin. The findings of these models, with the findings in the Rb model, may shed light on why p27 is a prognostic indicator. Isn't that what it is all about?

CONCLUSION

The aforementioned arguments are built on many assumptions, many of which run contrary to the general belief. However, what is unarguable is: 1) that p27 is a strong prognostic indicator, 2) that p27 status is not correlated with proliferation, and 3) that p27 participates in the withdrawal of cells from the cell cycle in response to differentiation-inducing signals. Furthermore, mice now exist where the prognostic indication of p27 is recapitulated in cancers: for Rb+/– mice in the pituitary, for Pten+/– mice in the prostate, and for inhibin–/– mice in the gonadal tissues.

What is important now, is to decide why low-p27 is prognostic: does it reflect enhanced mitogenic signals, the escape from anti-mitogenic signals, or an escape from senescence *(85)*. When we answer this question, low-p27 expression could join in the pantheon of useful markers. At this time, the three events all coordinate or impact on many levels, not the least of which is the sacrosanct gate-keeper of cell cycle, pRb. However, extrapolation of the ras-mediated changes espoused by Hanahan and Weinberg *(33)*, would be consistent with the notion that ras = mitogen, low-p27 = anti-mitogen, and p53-mdm2-arf-cyclin D-Rb may be related to apoptosis and senesence and the integration of the three events.

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