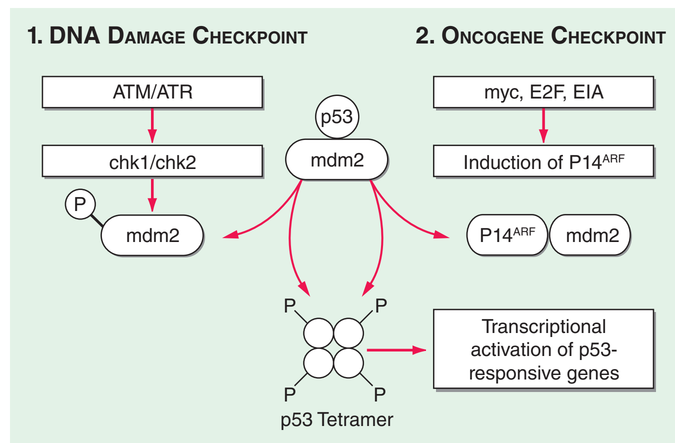

FIGURE 102e-1 Induction of p53 by the DNA damage and oncogene checkpoints. In response to noxious stimuli, p53 and mdm2 are phosphorylated by the ataxia-telangiectasia mutated (ATM) and related (ATR) serine/threonine kinases, as well as the immediate downstream checkpoint kinases, Chk1 and Chk2. This causes dissociation of p53 from mdm2, leading to increased p53 protein levels and transcription of genes leading to cell cycle arrest ($p21^{Cip1/Waf1}$) or apoptosis (e.g., the proapoptotic Bcl-2 family members Noxa and Puma). Inducers of p53 include hypoxemia, DNA damage (caused by ultraviolet radiation, gamma irradiation, or chemotherapy), ribonucleotide depletion, and telomere shortening. A second mechanism of p53 induction is activated by oncogenes such as *Myc*, which promote aberrant G_1/S transition. This pathway is regulated by a second product of the Ink4a locus, $p14^{ARF}$ (p19 in mice), which is encoded by an *a*lternative *r*eading *f*rame of the same stretch of DNA that codes for $p16^{Ink4a}$. Levels of ARF are upregulated by *Myc* and E2F, and ARF binds to mdm2 and rescues p53 from its inhibitory effect. This *oncogene checkpoint* leads to the death or senescence (an irreversible arrest in G_1 of the cell cycle) of renegade cells that attempt to enter S phase without appropriate physiologic signals. Senescent cells have been identified in patients whose premalignant lesions harbor activated oncogenes, for instance, dysplastic nevi that encode an activated form of BRAF (see below), demonstrating that induction of senescence is a protective mechanism that operates in humans to prevent the outgrowth of neoplastic cells.

detected, the p53 pathway is normally activated. Called the guardian of the genome, p53 is a transcription factor that is normally present in the cell in very low levels. Its level is generally regulated through its rapid turnover. Normally, p53 is bound to mdm2, a ubiquitin ligase, that both inhibits p53 transcriptional activation and also targets p53 for degradation in the proteasome. When damage is sensed, the ATM (ataxia-telangiectasia mutated) pathway is activated; ATM phosphorylates mdm2, which no longer binds to p53, and p53 then stops cell cycle progression, directs the synthesis of repair enzymes, or if the damage is too great, initiates apoptosis of the cell to prevent the propagation of a damaged cell **(Fig. 102e-1)**.

A second method of activating p53 involves the induction of $p14^{ARF}$ by hyperproliferative signals from oncogenes. $p14^{ARF}$ competes with p53 for binding to mdm2, allowing p53 to escape the effects of mdm2 and accumulate in the cell. Then p53 stops cell cycle progression by activating CDK inhibitors such as p21 and/or initiating the apoptosis pathway. Not surprisingly given its critical role in controlling cell cycle progression, mutations in the gene for p53 on chromosome 17p are found in more than 50% of human cancers. Most commonly these mutations are acquired in the malignant tissue in one allele and the second allele is deleted, leaving the cell unprotected from DNA-damaging agents or oncogenes. Some environmental exposures produce signature mutations in p53; for example, aflatoxin exposure leads to mutation of arginine to serine at codon 249 and leads to hepatocellular carcinoma. In rare instances, p53 mutations are in the germline (Li-Fraumeni syndrome) and produce a familial cancer syndrome. The absence of p53 leads to chromosome instability and the accumulation of DNA damage including the acquisition of properties that give the abnormal cell a proliferative and survival advantage. *Like Rb dysfunction, most cancers have mutations that disable the p53 pathway*. Indeed, the importance of p53 and Rb in the development of cancer is underscored by the neoplastic transformation mechanism of human papillomavirus. This virus has two main oncogenes, E6 and E7. E6 acts to increase the rapid turnover of p53, and E7 acts to inhibit Rb function; inhibition of these two targets is required for transformation of epithelial cells.

Another cell cycle checkpoint exists when the cell is undergoing division, the spindle checkpoint. The details of this checkpoint are still being discovered; however, it appears that if the spindle apparatus does not properly align the chromosomes for division, if the chromosome number is abnormal (i.e., greater or less than $4n$), or if the centromeres are not properly paired with their duplicated partners, then the cell initiates a cell death pathway to prevent the production of aneuploid progeny (having an altered number of chromosomes). Abnormalities in the spindle checkpoint facilitate the development of aneuploidy. In some tumors, aneuploidy is a predominant genetic feature. In others, a defect in the cells' ability to repair errors in the DNA due to mutations in genes coding for the proteins critical for mismatched DNA repair is the primary genetic lesion. This is usually detected by finding alterations in repeat sequences of DNA (called microsatellites) or microsatellite instability in malignant cells. In general, tumors either have defects in chromosome number or microsatellite instability, but not both. Defects that lead to cancer include abnormal cell cycle checkpoints, inadequate DNA repair, and failure to preserve genome integrity.

Efforts are under way to therapeutically restore the defects in cell cycle regulation that characterize cancer, although this remains a challenging problem because it is much more difficult to restore normal biologic function than to inhibit abnormal function of proteins driving cell proliferation, such as oncogenes.

CANCER AS AN ORGAN THAT IGNORES ITS NICHE

The fundamental cellular defects that create a malignant neoplasm act at the cellular level. However, that is not the entire story. Cancers behave as organs that have lost their specialized function and stopped responding to signals that normally limit their growth. Human cancers usually become clinically detectable when a primary mass is at least 1 cm in diameter—such a mass consists of about 10^9 cells. More commonly, patients present with tumors that are 10^{10} cells or greater. A lethal tumor burden is about 10^{12} to 10^{13} cells. If all tumor cells were dividing at the time of diagnosis, patients would reach a lethal tumor burden in a very short time. However, human tumors grow by Gompertzian kinetics—this means that not every daughter cell produced by a cell division is itself capable of dividing. The growth fraction of a tumor declines exponentially with time. The growth fraction of the first malignant cell is 100%, and by the time a patient presents for medical care, the growth fraction is 2–3% or less. This fraction is similar to the growth fraction of normal bone marrow and normal intestinal epithelium, the most highly proliferative normal tissues in the human body, a fact that may explain the dose-limiting toxicities of agents that target dividing cells.

The implication of these data is that the tumor is slowing its own growth over time. How does it do this? The tumor cells have multiple genetic lesions that tend to promote proliferation, yet by the time the tumor is clinically detectable, its capacity for proliferation has declined. We need to better understand how a tumor slows its own growth. A number of factors are known to contribute to the failure of tumor cells to proliferate in vivo. Some cells are hypoxemic and have inadequate supply of nutrients and energy. Some have sustained too much genetic damage to complete the cell cycle but have lost the capacity to undergo apoptosis and therefore survive but do not proliferate. However, an important subset is not actively dividing but retains the capacity to divide and can start dividing again under certain conditions such as when the tumor mass is reduced by treatments. Just as the bone marrow increases its rate of proliferation in response to bone