Beyond the Oncogene Revolution: Four New Ways to Combat Cancer

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It has become clear that tumorigenesis results from much more than just the activation of an oncogene and/or the inactivation of a tumor-suppressor gene, and that the cancer cell genome contains many more alterations than can be specifically targeted at once. This observation has led our group to a search for alternative ways to kill cancer cells (while sparing normal cells) by focusing on properties unique to the former. We have identified four approaches with the potential to generate new anticancer therapies: combatting the tactics by which cancers evade antitumor immune responses, targeting metabolic adaptations that tumor cells use to survive conditions that would kill normal cells, manipulating a cancer cell's response to excessive oxidative stress, and exploiting aneuploidy. This review describes our progress to date on these fronts.

Work over the past several years has made it clear that numerous alterations are involved in the malignant transformation of a normal cell into a cancer cell, much more than the mere activation of oncogenes and inactivation of tumor-suppressor genes. It is therefore too difficult to treat cancer by applying one or more agents each designed to target a specific mutational change. Instead, our group (and others) has investigated several promising novel approaches that target an aspect of the overall transformed state of a cancer cell. These aspects of transformation include the ability of tumors to evade immune responses mounted against them, the altered metabolic pathways tumors use to thrive under conditions that would kill normal cells, and the unusual mechanisms cancer cells use to maintain genomic stability in the face of hurdles such as excessive oxidative stress and aneuploidy. Manipulation of these altered cellular states may open up exciting new avenues for cancer therapy that do not depend on the targeting of an individual oncogene or tumor-suppressor gene. This review summarizes some of our work on the exploration of these intriguing new possibilities for cancer therapies.

COMBATING TUMOR EVASION TACTICS

Immunotherapy has become one of the most promising new strategies for fighting cancer. Although the immune system works every day to prevent incipient tumor cells from establishing, clearly the system fails often enough that cancers develop. Moreover, tumor cells deploy numerous tactics to evade and neutralize the immune responses they provoke. Thus, cancer immunologists have devised ways to stimulate specific components of the immune system to increase cancer cell killing and to reverse the evasive actions of cancer cells, rendering them once more vulnerable to immune attack.

Unleashing Antitumor T-Cell Responses

Cancers frequently express non-self-antigens that are recognized by a host's immune system. The binding of a complex containing a major histocompatibility complex (MHC) molecule bound to antigenic peptide (in this context, derived from the tumor) to a T cell's T-cell receptors (TCRs) is the first step in activating that T cell. The second step is co-stimulation in the form of binding between the T cell's CD28 molecules and B7 molecules expressed by the antigen-presenting cell (APC) bearing the peptide–MHC complex. Only upon successful co-stimulation is full T-cell activation achieved and an immune response mounted. However, to prevent excessive damage to normal tissues, any T-cell response is naturally limited in duration and magnitude by negative regulation exerted by other molecules up-regulated on the surface of activated T cells, including PD-1 and CTLA-4. Both PD-1 and CTLA-4 bind to B7 molecules with much higher affinity than does CD28, interrupting the co-stimulation mediated by this molecule and consequently shutting down the proliferation and IL-2 secretion of activated T cells. Although helpful in normal tissues, in the case of cancer, this PD-1- or CTLA-4-mediated inhibition may turn off an antitumor immune response before it has completed its job of eliminating cancer cells. Thus, researchers have sought a means of neutralizing this negative regulation so as to sustain anticancer immune responses and maintain the attack on the tumor.

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Several groups have independently developed monoclonal antibodies that block PD-1 or CTLA-4 from binding to CD28, creating an "immune blockade" that prevents the shutting down of the antitumor T-cell response and allows T cells to continue their assault on cancer cells (Page et al. 2014). These landmark experiments have led to the now-established anti-CTLA-4 and anti-PD-1 therapies that are currently used for the treatment of end-stage melanoma patients. Clinical trials are under way to evaluate these therapies in several other cancer types, including ovarian cancer, non–small cell lung carcinoma (NSCLC), colorectal cancer, renal cell carcinoma (RCC), metastatic hormone-refractory prostate cancer, and gastric cancer (Page et al. 2014).

Overcoming Anergy

So-called tumor-specific antigens (TSAs) expressed by cancer cells are often present either because of modification of a host's self-antigen or atypical expression of an unmodified self-antigen. These antigens will not usually trigger an immune response because the central and peripheral immune tolerance mechanisms designed to prevent autoimmunity will have resulted in the elimination of T cells responding to the original self-antigens (Ohashi 2003). Surviving T cells that do recognize such TSAs are usually rendered inactive by the induction of anergy (a state of nonresponsiveness) (Willimsky et al. 2008). There is some evidence in cancer patients that anergy of antitumor T cells can also be induced by the tumor itself, and that the transcription factor Egr2 may be involved in controlling such T-cell anergy (Safford et al. 2005; Zheng et al. 2012; Pardoll 2015). However, the downstream molecular mechanisms involved in establishing and maintaining the anergic state are incompletely understood. Moreover, the lack of a specific surface marker identifying an anergic T cell makes this line of research a difficult challenge. Nevertheless, if anergy can be reversed, as by the appropriate delivery of a second stimulatory signal, any TSA-specific T cells present might become activated and mount an effective antitumor response (Pellegrini et al. 2010).

Restoring Priming and Innate Responses

The molecules delivering co-stimulatory signals to T cells are up-regulated on the surfaces of APCs such as dendritic cells (DCs) after these cells use their Toll-like receptors (TLRs) to sense microbial products and become activated. This engagement of TLRs and initiation of DC maturation also contributes to activation of the innate immune system. In the case of a tumor that expresses no non-self-antigens that could trigger TLR binding and DC maturation, the innate immune response is limited and few cytokines and chemokines are produced. As a result, the recruitment of innate and adaptive immune cells able to attack tumor cells does not occur, and the cancer is allowed to progress. Several groups have experimented with the application of endogenous factors known to boost DC maturation in vitro, including heat shock proteins, uric acid, and damage-associated molecular patterns (DAMPs) such as high-mobility group box 1 protein (HMGB1) (Tesniere et al. 2008). In vivo chemotherapy increases the abundance of these factors due to increased cell death, leading to a more robust immune response (Pellegrini et al. 2010).

Overcoming the Negative Influence of the Tumor Microenvironment

Mechanisms designed to prevent autoimmunity in healthy individuals can compromise anticancer immune responses when they take effect in the tumor microenvironment (TME). These mechanisms include the actions of T regulatory (Treg) cells, myeloid-derived suppressor cells (MDSCs), and other inhibitory cell types (Rabinovich et al. 2007; Murdoch et al. 2008; Pittet 2009). Furthermore, cancer cells actively influence the TME to prevent or dampen antitumor immune responses by expressing negative regulatory ligands such as PDL1 or B7H4. The binding of these regulators to their cognate receptors on T cells inhibits the activity of these cells and impairs the adaptive antitumor response (Krambeck et al. 2006; Blank and Mackensen 2007). In addition, tumor cells actively secrete immunosuppressive signaling molecules and cytokines such as interleukin-10, transforming growth factor-β, and/or indoleamine 2,3-dioxygenase (IDO) (Katz et al. 2008; Mantovani et al. 2008). Treatment with monoclonal antibodies that can block these regulatory cells and mechanisms might therefore significantly enhance antitumor responses (Pellegrini et al. 2010). Indeed, newly devised immunotherapy drugs such as ipilimumab release these brakes, enabling the immune system to keep up the attack (Crouch 2016).

Preventing Immune Escape

Although the killing of tumor cells by cytolytic $CD8^+$ T cells may initially reduce a cancerous mass, it may eventually promote the selective outgrowth of tumor cells that have lost or mutated the antigens targeted by those T cells, blunting the response. This selective pressure may also cause the tumor cell to alter its MHC expression or antigen processing, again abetting immune escape. Therefore, strategies such as local radiotherapy, inhibition of histone deactylase and/or DNA methyltransferase, or stimulation with type I interferons can enhance levels of MHC expression and antigen presentation on tumor cells (Pellegrini et al. 2010). In addition, natural killer cells that target tumor cells lacking surface MHC can be harvested from a cancer patient, expanded and activated ex vivo, and then given back to the patient to boost antitumor activity (Chan et al. 2008).

Penetrating the Wall

All of the above tumor properties can counter responses mediated by T cells and/or permit the cancer cells to

act like stealth bombers and hide from the immune system. However, recent work has revealed that nefarious co-opting of immune cells in the service of the cancer also exists. For example, analysis of human papillomavirus (HPV)-associated head and neck squamous cell carcinomas revealed the colocalization of tumor-associated macrophages, PDL-1-expressing tumor cells, and $CD8^+$-infiltrating lymphocytes expressing high levels of PD-1 in a "wall" blocking access to the tumor's interior (Lyford-Pike et al. 2013). This cell collection creates an immune-privileged site that allows the tumor cells to shut down the responses of any T cells already in the cancerous mass and to resist the penetration of any new T cells (Lyford-Pike et al. 2013). Some have likened the outer layer of macrophages in this wall to the fortifications that prevented the Greek soldiers from marching into Troy to rescue Helen (Apple 2016). The search for a microscopic Trojan horse that can break through or slither over the wall and expose the tumor cells to fresh immune attack is under way.

ALTERED METABOLIC PATHWAYS

Warburg Was Right

In 1924, Otto Warburg postulated that, "The cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar" (Warburg et al. 1924; Koppenol et al. 2011). This hypothesis was subsequently dubbed the "Warburg effect." In 1966, Warburg repeated his theory that cancer is caused mainly by a switch from normal cell respiration to fermentation, and that this switch might be induced by damage to the enzymes needed for respiration (http://www.mediatheque.lindau-nobel.org/videos/31517/on-the-primary-causes-and-on-the-secondary-causes-of-cancer-german-presentation-1966/laureate-warburg). Many scientists are now coming around to the view that Warburg was indeed right, and that cancer cells undergo metabolic changes that might make them vulnerable to new types of anticancer treatment.

Altered Metabolism and Reactive Oxygen Species Handling

Cells undergoing malignant transformation show certain metabolic adaptations (in addition to their genetic and epigenetic changes) that may be induced by their altered TME. The signaling pathways that are up-regulated in the course of these adaptations are not intrinsically tumorigenic but permit incipient tumor cells to thrive under conditions killing normal cells (Galluzzi et al. 2013). Tumor cell metabolism is altered to maximize the addressing of the fundamental needs of proliferating cells: increased macromolecule biosynthesis, rapid ATP production to generate energy, and heightened regulation of intracellular redox status. Alterations to carbohydrate, nucleic acid, lipid, and protein metabolism ensue in the cancer cell's fight to survive and divide (Cairns et al. 2011). These changes cause precancerous and cancerous cells to become metabolically "addicted," meaning that new avenues of therapeutic intervention may abound. Because normal cells do not endure the same degree of energy stress as frantically dividing malignant cells, an agent targeting a metabolic adaptation present only in tumor cells might spare normal cells, reducing deleterious side effects.

In addition to nimble metabolism, the regulation of reactive oxygen species (ROS) is crucial for the survival and functions of tumor cells. The increased growth of cancer cells produces increased ROS, so that tumor cells have to alter their signaling pathways mediating ROS regulation to cope. Elevated ROS in cancers are generated by endoplasmic reticulum (ER) stress, defective metabolism, hypoxia, and oncogene activity (Gorrini et al. 2013b). At the biochemical level, such ROS are routinely neutralized via glutathione or NADPH and by dietary antioxidants. Cells under stress conditions also activate transcription factors such as NRF2 that drive the expression of antioxidant genes. The activities of tumor suppressors such as PTEN, p53, BRCA1, and ATM are also increased in stressed cells (Gorrini et al. 2013b). Transformation itself is a form of stress, and so as ROS increase in a precancerous or cancerous cell, it up-regulates its antioxidant pathways to protect itself from ROS-induced death.

Although antioxidants have been proposed as anticancer agents, there is controversy over their role in tumorigenesis. In a recent study in our laboratory, we showed that synthesis of the antioxidant glutathione (GSH), which requires the activity of the modifier subunit of glutamate-cysteine ligase (GCLM), is in fact necessary for cancer initiation (Harris et al. 2015). Targeted therapeutics that block antioxidant pathways may thus paradoxically be helpful because they induce the apoptotic death of tumor cells. Alternatively, a drug that could increase ROS production above the level that the cancer cell's up-regulated antioxidant mechanisms could handle might kill the tumor cell. Once again, because these antioxidant pathways are not activated in resting normal cells, they would in theory be unaffected by the drug.

Mutant IDH1 and IDH2

Much recent research in the metabolic adaptation field has been focused on isocitrate dehydrogenases (IDHs) 1 and 2, which usually influence the cytoplasmic/mitochondrial NADP:NADPH ratio and thus a cell's reducing potential (Cairns and Mak 2013). Cancer-associated mutations in *IDH1* were identified through cancer cell genome sequencing efforts (Parsons et al. 2008; Mardis et al. 2009) and were followed by the discovery of similar mutations in *IDH2* (Yan et al. 2009). The normal biochemical function of IDH1/2 is to convert isocitrate to α-ketoglutarate and concomitantly reduce NADP to NADPH while producing CO_2 (Cairns and Mak 2013). IDH1 carries out this reaction in the cytoplasm, whereas IDH2 does so in the mitochondria (Fig. 1). Cancer-associated *IDH1* mutations predominantly alter arginine-132 (R132) in the enzyme's active site, whereas those altering

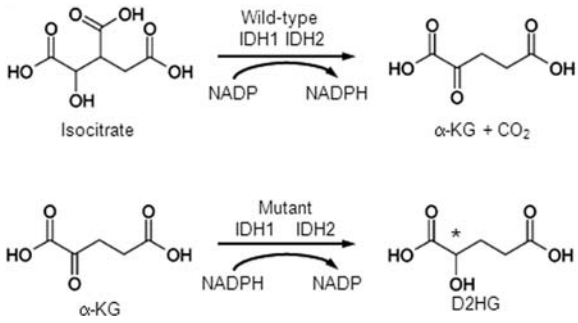

Figure 1. Enzymatic reactions catalyzed by wild-type and mutant IDH enzymes. (*Top*) Wild-type IDH1 and IDH2 convert isocitrate to α-KG and CO_2, with concomitant production of NADPH from NADP. (*Bottom*) Tumor-associated mutant IDH1 and IDH2 enzymes convert α-KG to D2HG, with concomitant production of NADP from NADPH. D2HG is a chiral molecule very similar in structure to α-KG. The chiral center in D2HG is denoted by *. (Adapted from Cairns and Mak 2013.)

IDH2 usually occur at R172 and R140 (Ward et al. 2010; Cairns and Mak 2013). However, rather than removing IDH activity, the R132, R172, and R140 mutations of IDH1/2 give these altered enzymes a new function. In 2009, researchers at Agios Pharmaceuticals used a metabolite profiling strategy to show that the normal IDH product α-ketoglutarate is converted by mutated IDH1/2 to 2-hydroxyglutarate (D2HG) in a reaction that consumes, rather than produces, NADPH (Fig. 1; Dang et al. 2009; Cairns and Mak 2013). D2HG is now viewed by many in the field to be an oncometabolite.

IDH1 mutations have been found at relatively high frequency in acute myeloid leukemia (AML) (Mardis et al. 2009), cholangiocarcinoma (Borger et al. 2012; Wang et al. 2013), glioblastoma multiforme (GBM) (Parsons et al. 2008), and chondrosarcoma (Amary et al. 2011). Some melanomas and colon, prostate, and NSCLC cancers also possess mutated *IDH1* genes (Kang et al. 2009). *IDH2* mutations occur in AML (Mardis et al. 2009), cholangiocarcinoma (Borger et al. 2012; Wang et al. 2013), myelodysplastic syndrome (MDS), and myeloproliferative disorder (MPD) (Patnaik et al. 2012; Rakheja et al. 2012; Shih et al. 2012), D2HG aciduria (Kranendijk et al. 2010), angioimmunoblastic T-cell lymphoma (AITL) (Cairns et al. 2012), and chondrosarcoma (Amary et al. 2011).

Mouse Models of Mutated IDH1/2

To generate accurate mouse models of oncogenesis related to IDH mutations, we used the lox–stop–lox (LSL) system to create conditional knock-in (KI) mice in which Cre recombinase drives the excision of the stop codon upstream of *IDH1* exon 4. The mutated IDH1 protein is then expressed from the endogenous locus (Sasaki et al. 2012a,b). Without Cre, neither the wild-type (WT) *IDH* allele nor the LSL IDH1 R132 mutated allele is expressed. It turns out that WT *IDH1* function is not necessary for mouse survival under laboratory conditions because complete IDH1 knockout mice are fertile and viable (Cairns and Mak 2013). Mice heterozygous for the *IDH1* R132 KI allele that also constitutively express Cre do not survive beyond the early embryonic stage, suggesting that the D2HG produced by the mutated IDH1 enzyme is lethal during development (Cairns and Mak 2013). When we crossed LysM-Cre mice with our IDH1 KI mice to examine the effect on the myeloid compartment, the mutant mice were born normally and grew as expected until 6 months of age, when they began to show evidence of extramedullary hematopoiesis, splenomegaly, and reduced bone marrow cellularity (Sasaki et al. 2012b). Because tumor cells in human AML and glioma patients with mutated IDH1 or IDH2 show altered DNA methylation (Figueroa et al. 2010; Noushmehr et al. 2010), we examined DNA methylation in our IDH1 KI mice. An increased proportion of hypermethylated CpG sites was present in LSK cells of LysM-Cre IDH1 KI mice, with a significant increase in CpG sites showing $>80\%$ methylation (Sasaki et al. 2012b). Moreover, LysM-Cre IDH1 KI cells showed alterations to DNA methylation and histone hypermethylation reminiscent of those in human IDH-mutant AML (Sasaki et al. 2012b).

Involvement of ATM and Notch1

Our most recent work has shown that the *Atm* gene is down-regulated in IDH1 KI mice as a consequence of increased histone methylation and the closing of the chromatin structure (Inoue et al. 2016). By using CyTOF mass cytometry to identify proteins that were differentially expressed between WT and IDH-KI mice, we showed that ATM was significantly underexpressed in hematopoietic stem cells (HSCs) of the mutant animals. The *Atm* promoter in these mutant cells showed an accumulation of methylated histone H3K9 and a closed chromatin structure (Inoue et al. 2016). Furthermore, the number of long-term hematopoietic stem cells (LT-HSCs) in these mice was reduced and the self-renewal capacity of these cells was impaired. In addition to their decreased ATM expression, IDH1-mutant LT-HSCs showed increased sensitivity to DNA damage as measured by the formation of 53BP1 and γH2AX foci, regardless of whether this damage was spontaneous or induced by irradiation. Importantly, all these effects were found to be independent of TET2 (Inoue et al. 2016). Our findings provide mechanistic insight into why IDH1 KI mice develop myeloid malignancies. Significantly, like our mutant mice, patients with IDH-mutated AML show low expression levels of ATM and other DNA damage repair-associated genes (Inoue et al. 2016; Penard-Lacronique and Bernard 2016).

We have also introduced the IDH1 R132 mutation into the entire hematopoietic system by crossing our IDH1 KI mice with Vav-Cre animals, and we have investigated the role of IDH1 mutations in T-cell malignancies (Hao et al. 2016). Vav-Cre IDH1-KI mutants spontaneously developed T-cell acute lymphoblastic leukemia (T-ALL) that was transplantable to fresh recipient mice and that maintained its mutant IDH1 expression (Hao et al. 2016).

When we performed whole-exome sequencing on isolated tumors, we identified a spontaneous activating mutation in Notch1, a gene commonly mutated in human T-ALL (Ferrando 2009). These results suggested that IDH1 mutations may have the capacity to cooperate with Notch1 mutation to drive T-ALL. Crossing our Vav-Cre IDH1 mutants with conditional Trp53 null mice accelerated the onset of T-cell lymphomagenesis (Hao et al. 2016). Interestingly, metabolomics analysis revealed that tumor cells derived from these double mutants showed increased dependence on both glucose and glutamine. Thus, mutant IDH1 may contribute to malignancy in the T-cell lineage and alter the metabolic profile of these cells (Hao et al. 2016).

Oncogenicity of D2HG

Our studies of IDH1/2-mutated and -related mutant mice have reinforced our hypothesis that it is the novel activity of these mutant IDH enzymes (resulting in massive D2HG production) that is the basis of their tumorigenicity. But why is D2HG oncogenic? D2HG competitively inhibits a family of 2-OG-dependent dioxygenases that all use α-ketoglutarate as a substrate (Cairns and Mak 2013). Mammalian cells contain more than 60 2-OG-dependent dioxygenases contributing to biologic processes as diverse as fatty acid metabolism, collagen biosynthesis, hypoxia sensing, modifications of chromatin and RNA, and DNA repair (Rose et al. 2011). In general, these enzymes convert α-ketoglutarate to succinate and CO_2 in a reaction requiring ascorbate, iron, and oxygen as cofactors (Rose et al. 2011). Competitive inhibition of 2-OG-dependent dioxygenases by D2HG has been shown in vitro (Chowdhury et al. 2011), and the increased levels of D2HG in IDH-mutant tumors imply that D2HG can also impair the activities of 2-OG-dependent dioxygenases in vivo (Cairns and Mak 2013). Potential targets of D2HG-mediated inhibition include the JumonjiC domain–containing histone demethylases, the TET proteins involved in DNA methylation, the prolyl hydroxylases (PHDs) that regulate hypoxia-inducible factor (HIF) signaling, and the PHD and lysyl hydroxylases (LHDs) required for collagen maturation (Cairns and Mak 2013). Work is ongoing to determine exactly how D2HG-mediated inhibition of any or all of these enzymes leads to malignancy.

TARGETING REACTIVE OXYGEN SPECIES

As noted above, ROS are key intracellular stress factors that are elevated in cancer cells. Our group has engaged in extensive study of why excess ROS are oncogenic. We have shown that BRCA1 deletion increases ROS in breast cancer cells because the lack of BRCA1 impairs the Nrf2-mediated antioxidant response (Gorrini et al. 2013a). BRCA1 normally interacts directly with Nrf2 to influence Keap1-mediated Nrf2 ubiquitination and thus Nrf2 activation and stability (Gorrini et al. 2013a). A critical finding has been that estrogen treatment partly restores Nrf2 activity in mammary tumor cells lacking BRCA1, protecting them from excessive oxidative stress and furthering their growth (Gorrini et al. 2013a, 2014). These results provide the long-sought explanation for why it is almost exclusively breast and ovarian tumors that arise in carriers of *BRCA1* mutations (Fig. 2). We hypothesize that somatic loss of BRCA1 function in heterozygous carriers of *BRCA1* mutations has tissue-specific effects. In tissues lacking high levels of estrogen, BRCA1 deficiency blocks Nrf2 antioxidant signaling such that ROS accumulate to levels that kill the BRCA1-deficient cells.

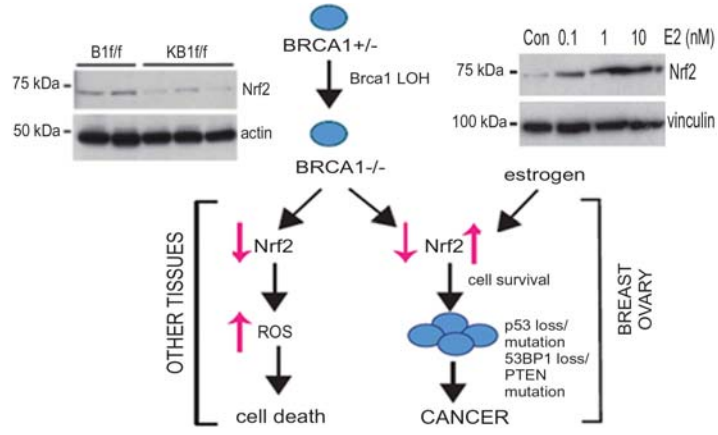

Figure 2. Model of the role of NRF2 regulation in BRCA1-associated tumorigenesis. (*Top left*) Immunoblot comparing levels of the antioxidant transcription factor Nrf2 in mammary epithelial cells of control mice (B1f/f) and double-mutant BRCA1- and P53-deficient mice (KB1f/f). Nrf2 is decreased in the absence of BRCA1. (*Top right*) Immunoblot demonstrating the induction of Nrf2 in wild-type (WT) mammary epithelial cells treated with increasing doses of estrogen (E2). (*Center*) When the function of the WT BRCA1 allele is lost in a heterozygous carrier of a BRCA1 mutation, the outcome depends on the tissue type. In breast and ovary, where estrogen is abundant, NRF2 is up-regulated so that incipient tumor cells survive to acquire additional mutations, such as loss of p53 or PTEN function, and eventually undergo full transformation. In other tissues lacking high levels of estrogen, NRF2 activity is decreased in the absence of BRCA1. The excessive ROS (reactive oxygen species) generated in the incipient cancer cell cannot be combated effectively and the cell dies, preventing tumor formation. LOH, loss of heterozygosity. (Adapted from Gorrini et al. 2014.)

Tumor cells do not get a chance to develop. However, in the estrogen-rich environment of mammary and ovarian tissues, Nrf2 can be activated by a PI3K–AKT-dependent mechanism even in the absence of BRCA1. The BRCA1-deficient incipient tumor cells are protected against oxidative stress-induced death and survive to acquire additional oncogenic mutations. If such a BRCA1-deficient cell sustains loss of PTEN function, the PI3K–AKT pathway may run uncontrolled and further stimulate estrogen-driven NRF2 signaling. Pathways activated downstream from AKT, including those with antioxidant or mitogenic effects, may combine with the genomic instability induced by the failure of BRCA1-mediated DNA repair to ultimately drive malignant transformation of the BRCA1-deficient cells (Gorrini et al. 2014).

TARGETING ANEUPLOIDY

A long-term goal of our group is to come up with new classes of anticancer therapies that may be more effective for certain cancers than existing agents. As noted above, no one approach is likely to eliminate all types of malignancies because of the extent of variation in cancer cell genomes. Our latest efforts have targeted aneuploidy, an alteration present in most advanced cancer cells but absent from normal cells.

We systematically combined gene expression analysis with RNAi screening of human breast tumor samples and cancer cell lines that show aneuploidy. Polo-like kinase-4 (PLK4), an enzyme crucial for the maintenance of aneuploidy, was identified as a promising target (Mason et al. 2014). Our academic drug discovery team isolated a selective and potent small molecule inhibitor of PLK4 called CFI-400945 (Mason et al. 2014). Before they died, cancer cells treated with CFI-400945 showed centriole duplication and mitotic defects similar to those resulting from PLK4 kinase inhibition in vitro (Mason et al. 2014). Mice that bore xenografted human ovarian or breast cancer tissues and were treated with CFI-400945 showed significant tumor growth inhibition, which was influenced by the PTEN status of the cancer cells (Mason et al. 2014). Xenografts lacking PTEN function were more responsive to CFI-400945 treatment than were those with WT PTEN (Mason et al. 2014). Thus, PTEN status may be a predictive biomarker for our new anticancer agent.

CFI400945 recently entered a first-in-human phase I trial to establish its safety, tolerability, and pharmacokinetics (PK) and to determine the recommended phase II dose (RP2D) (Bedard et al. 2016). For this trial, 31 patients with advanced solid tumors of any type were enrolled between April 2014 and December 2015. The trial followed a standard 3+3 dose escalation design, with a starting dose of 3 mg delivered orally once daily. The primary end point was the incidence of dose-limiting toxicities (DLTs). Over the course of the trial, dosing reached 72 mg without observation of any DLT events. The most frequent treatment-related adverse events (trAEs) were fatigue (24%), diarrhea (17.2%), nausea (17.2%), decreased appetite (13.8%), and vomiting (6.9%), all of grade 1 or grade 2 in severity (Bedard et al. 2016). To date, two patients enrolled at the 48 mg dose level have completed >6 cycles, including a patient with KRAS mutant colorectal cancer who achieved a 24% reduction in target lesions and >50% reduction in serum carcinoembryonic antigen (CEA) levels (Bedard et al. 2016). Our data so far indicate that CFI-400945 is well tolerated at doses up to 72 mg and has a favorable PK profile. Most importantly, preliminary evidence of effective antitumor activity has been observed. Exploration of 96 mg daily dosing of CFI400945 is ongoing (Bedard et al. 2016).

CONCLUSION

We believe that the future looks bright for the development of new types of anticancer therapies that can truly make a difference for cancer patients. We now recognize that cancer differs in its genetics from individual to individual, and that no one strategy focusing on tumor-associated mutations will cure all. Approaches that can boost the body's antitumor responses, shut down the tumor's escape mechanisms, or undermine its metabolic or replicative adaptations have the potential to help many sufferers of this dreaded disease.

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