(Zhang et al., 2011), in medulloblastoma high nuclear β-catenin is an established predictor of increased patient survival (Ellison et al., 2005). How, then, does a β-catenin/FoxM1 interaction function in medulloblastoma, if it exists at all? Do mutations to β-catenin at key regulatory residues, which are common in medulloblastoma, alter the armadillo repeat-mediated interaction of β-catenin with FoxM1? It will be interesting to see what a more thorough examination of the FoxM1/β-catenin interaction in medulloblastoma can elucidate concerning this discrepancy.

A further point that the current study (Zhang et al., 2011) highlights is the potential for a widespread interaction between Fox proteins and the Wnt/β-catenin signaling pathway. Previous studies in *C. elegans* and mammalian cells demonstrated a physical interaction between β-catenin and multiple FOXO proteins (Essers et al., 2005). Similar to the results of Zhang et al. (2011), these interactions were mediated by the armadillo repeats of β-catenin and the portion of the FOXO protein containing the forkhead DNA-binding domain (Essers et al., 2005). Additionally, there is precedence for Wnt/β-catenin signaling in the regulation of Fox proteins. This is the case for FoxN1, the protein that is mutated in nude mice, resulting in athymic and hairless animals (Balciunaite et al., 2002). In the thymus and the hair follicle, Wnt/β-catenin pathway stimulation leads to activation of FoxN1, which possesses Wnt Response Elements (WREs) in its promoter (Balciunaite et al., 2002). In fact there may also be a reciprocal relationship between Wnt/β-catenin and FoxM1 because FoxM1 can directly bind to the promoter of human β-catenin and regulate its expression in endothelial cells. However, the frequency of cross-regulation between these two pathways, in both normal and tumor environments, remains to be seen. Additionally, whether FoxM1 can regulate β-catenin nuclear translocation in other cell contexts has yet to be established. In any case this study illuminates the potential for Wnt/β-catenin pathway control by an unusual source, FoxM1, and the role for these proteins in glioblastoma progression.

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RAIDDing ER Stress for Oncolytic Viral Therapy

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Oncolytic viruses exploit molecular differences between normal and cancer cells to selectively kill the latter. Results of a synthetic lethal screen described in this issue of *Cancer Cell* demonstrate that components of the unfolded protein response (UPR) limit virus-induced tumor cell killing and identify a strategy to utilize this knowledge.

Productive viral infection mimics oncogenic transformation in several respects, and some of the same molecular mechanisms are employed by viruses and cancer cells to disrupt key homeostatic mechanisms. These similarities serve as the foundation for the development of "oncolytic" viruses that are designed to specifically target and kill cancer cells (Parato et al., 2005). Although some targeting strategies involve engineering viruses so that they bind specifically to cancers, an even more attractive approach involves developing viruses that can only replicate in cancer cells that contain specific defects in homeostatic control. For example one of the products of the adenovirus E1B

locus is a protein that specifically disrupts p53 function, thereby undermining the host p53-dependent antiviral response that would otherwise result in inhibition of DNA synthesis and/or apoptosis (Debbas and White, 1993). Mutant forms of adenovirus that lack E1B 55K should only replicate in cells with defective p53 function, i.e., cancers. Several groups have developed E1B mutant adenoviruses for cancer therapy, and promising results have been obtained with several of them, including Onyx Pharmaceuticals' ONYX-015 (Khuri et al., 2000). Another promising approach exploits the presence of mutant active Ras (Parato et al., 2005).

Rhabdoviruses are RNA viruses that are also being developed as oncolytic agents. Their tumor selectivity is related largely to the fact that tumor cells are often resistant to the antiviral effects of type I interferons (IFNs), which can completely suppress viral replication in normal cells (Stojdl et al., 2000). Eliminating viral mechanisms that suppress autocrine IFN production enhances oncolytic activity while further reducing toxicity to normal host tissues (Stojdl et al., 2003).

In this issue of *Cancer Cell*, another novel strategy to promote their oncolytic effects has been uncovered (Mahoney et al., 2011). The investigators designed a synthetic lethal RNAi screen to identify cytoprotective pathways that limit tumor cell killing induced by the Maraba rhabdovirus in three different human cancer cell lines. Their "hits" were enriched for genes that function within two of the three major pathways that respond to endoplasmic reticular (ER) stress, commonly referred to as the unfolded protein response (UPR) (Figure 1). More specifically, the screen implicated the ATF6 and IRE1/XBP1 pathways (but not the PERK/eIF2α/ATF4 pathway), as well as downstream genes involved in the transport of protein aggregates out of the ER to the proteasome, in cytoprotection. Importantly, the group also identified a novel small molecule inhibitor of IRE1 that also sensitized tumor but not normal cells to the oncolytic effects of the virus in vitro and in xenografts. Therefore, if the inhibitor can be further optimized to increase its potency, there is a good chance that these preclinical observations can be translated in patients with cancer.

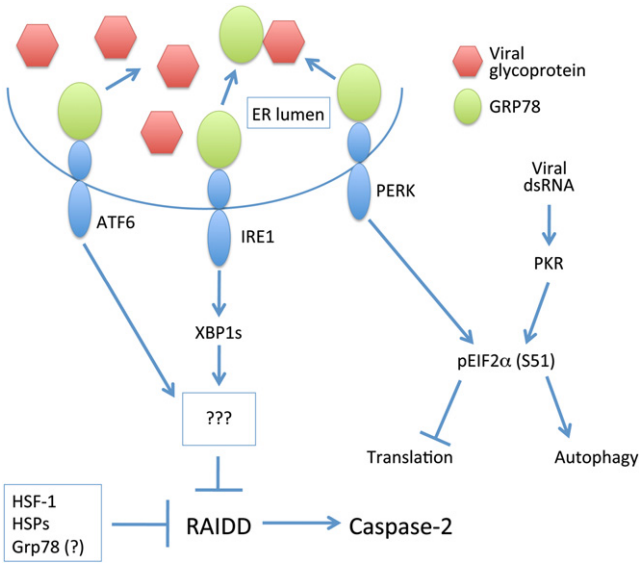

Figure 1. Viral Infection and the UPR

Under normal conditions the three major arms of the UPR (ATF6, IRE1, and PERK) are maintained in inactive states via interactions between their ER luminal domains and the ER chaperone, Grp78/BiP. Viral or misfolded proteins compete for binding to Grp78, allowing for activation of the UPR. Viral double-stranded RNA can also promote phosphorylation of eIF2α in normal cells by activating PKR. Knockdown of either ATF6 or IRE1/XBP1 results in upregulation of the caspase-2 adaptor protein, RAIDD, via unknown mechanisms, thereby "preconditioning" cells for virus-induced apoptosis. RAIDD-mediated caspase-2 activation appears to be controlled via mechanisms that involve the heat-shocked-responsive transcription factor, HSF-1, and perhaps heat-shocked proteins and other chaperones.

At first glance it might seem surprising that hits within the PERK/eIF2α arm of the UPR were not identified, but in fact this makes sense. Phosphorylation of eIF2α results in global downregulation of cap-dependent host translation, so viruses have evolved many different mechanisms to prevent eIF2α phosphorylation or its downstream consequences in normal cells (Mohr, 2005). Furthermore, we have observed that many tumor cells fail to display increased eIF2α phosphorylation or translational arrest in response to proteotoxic and ER stress (Zhu et al., 2010; unpublished data), so this arm of the UPR may be disabled in a large subset of cancers anyway. In these cancers the coupling between the proteasome and autophagy is disrupted (Zhu et al., 2010), which may also be advantageous for productive viral infection if autophagy plays some role in limiting it (Tallóczy et al., 2002).

One might also predict that knockdown of UPR or ER-associated decay (ERAD) components would cause a buildup of protein aggregates within the ER and that subsequent viral infection dramatically exacerbates the situation by overwhelming an already stressed ER-Golgi network with increased protein synthetic load. Indeed, UPR inhibition did cause features of ER stress in infected cells, but they resolved quickly and did not lead to an obvious increase in the accumulation of protein aggregates (Mahoney et al., 2011), strongly suggesting that the sensitization caused by pretreatment with UPR inhibitors was not caused by this mechanism. Rather, UPR inhibition appeared to "precondition" the cells to subsequent virus-induced cell death by upregulating expression of the caspase adaptor protein, RAIDD, and promoting activation of caspase-2, and knockdown of caspase-2 almost completely rescued the synthetic lethal interaction between UPR inhibition and viral infection. Recent work from Doug Green's group (Bouchier-Hayes et al., 2009) demonstrated that RAIDD-mediated caspase-2 activation is controlled by the stress-responsive transcription factor, HSF-1, suggesting that heat-shocked proteins and/or other (perhaps ER-based?) molecular chaperones may play central roles in controlling stress-induced caspase-2 activation (Figure 1). Left unresolved are the molecular mechanisms that link UPR inhibition to RAIDD upregulation and viral infection to caspase-2 activation. It does seem likely that some (possibly subtle) perturbation of protein aggregate clearance plays a role, but how, and especially why, this low-level stress, that appears to be completely resolved prior to viral infection, sets the stage for subsequent apoptosis awaits further investigation.

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Succination of Keap1 and Activation of Nrf2-Dependent Antioxidant Pathways in FH-Deficient Papillary Renal Cell Carcinoma Type 2

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Fumarate hydratase (FH) is a tumor suppressor, but how it acts is unclear. Two reports in this issue of *Cancer Cell* reveal that FH deficiency leads to succination of Keap1, stabilization of Nrf2, and induction of stress-response genes including *HMOX1*, which is important for the survival of FH-deficient cells.

The fumarate hydratase gene (*FH*) encodes a TCA cycle enzyme and functions as a tumor suppressor gene. Heterozygous germline *FH* mutations result in hereditary leiomyomatosis and renal cell cancer (HLRCC), a syndrome characterized by smooth muscle tumors and papillary renal cell carcinoma type 2 (pRCC-2) (Tomlinson et al., 2002). In tumors, the wild-type *FH* allele is lost, and FH function is abrogated. pRCC-2 tumors in patients with HLRCC tend to metastasize early, and currently, there is no therapy.

How FH suppresses tumor formation is unknown. FH loss causes fumarate accumulation in tumor cells, and fumarate is a competitive inhibitor of 2-oxoglutarate-dependent prolyl hydroxylase domain-containing proteins (PHD) that hydroxylate HIFα. When hydroxylated, HIFα is recognized by the pVHL E3 ubiquitin ligase complex and is degraded. Because *VHL* is frequently mutated in renal cancer leading to HIF stabilization, a model whereby HIF is upregulated in pRCC-2 as a consequence of PHD inhibition by fumarate is attractive. However, the importance of PHD inhibition and HIF stabilization in the development of FH-deficient tumors remains unknown.

Keap1 is the substrate recognition subunit of a Cul3-based E3 ubiquitin ligase complex that regulates Nrf2, a pivotal transcription factor in the antioxidant response. Keap1 proteins dimerize through an N-terminal BTB domain, which through an intervening region (IVR) is linked to a C-terminal DC domain that contains a β-propeller made up largely of kelch repeats (Figure 1A). Two β-propellers in a Keap1 dimer interact with an Nrf2 monomer. Nrf2 contains two different Keap1-interacting motifs. Binding through both motifs is required for Nrf2 degradation, which led to a "hinge and latch" model (Hayes et al., 2010; Taguchi et al., 2011). Under normal conditions, Keap1 promotes Nrf2 ubiquitylation and proteosomal-mediated degradation. However, in the presence of electrophiles or reactive oxygen species, Keap1 is modified at several reactive Cys residues, resulting in Nrf2 stabilization and the activation of a protective gene expression program that includes *HMOX1*, an archetypal stress response gene (Hayes et al., 2010).

In this issue of *Cancer Cell*, Ooi et al. (2011) and Adam et al. (2011) show that FH loss results in Keap1 inactivation and Nrf2-dependent activation of antioxidant pathways. Through gene expression analyses, both groups discovered that FH deficiency was associated with increased expression of antioxidant genes, and this was accompanied by the accumulation of Nrf2. Reconstitution of FH-deficient