investigators prioritize candidate genes for functional studies.

As a final step, the authors sought to determine to what degree murine gliomas might recapitulate gene expression subclasses of human glioblastoma described in prior studies (Phillips et al., 2006; Verhaak et al., 2010). Unsupervised hierarchical clustering analysis across *p53:Pten* and *p53:Pten:Rb1* tumors generated three clusters of murine gliomas, HC1, HC2, and HC3, with significant similarity to proneural and mesenchymal subclasses of GBM. One interesting deviation from the human data, however, was that correlation of the mouse histology and genetics with the murine expression subclasses was weak, yet such correlations are clearly present in human GBM, particularly for the mesenchymal subclass that is associated with *NF1* loss, necrosis, and inflammation. While further refinement of mouse-to-human expression correlations is likely required, the results support the idea that mouse glioma models could be very helpful in exploring the diversity of human GBM subclasses with implications for better diagnosis and prediction of prognosis in patients. What seems equally clear is that mouse models can still generate novel ways to surprise and inform investigators who seek to understand these most challenging cancers of the brain.

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Escaping Anoikis through ROS: ANGPTL4 Controls Integrin Signaling through Nox1

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Reactive oxygen species (ROS) mediate various cell fate decisions in normal and transformed cells. In this issue of *Cancer Cell*, Zhu et al. demonstrate the ability of ANGPTL4 to engage integrin-dependent survival signals by activation of the NADPH oxidase Nox1, thus mimicking anchorage conditions and bypassing anoikis by controlling ROS.

Cancer cells have long been known to display abnormal redox metabolism, releasing increased levels of reactive oxidants compared with normal cells. The exact significance of such reactive oxygen species (ROS) production as it pertains to malignant transformation, however, has been less clear. ROS cause oxidative stress, which results in mutations to both nuclear and mitochondrial DNA. With loss of fail-safe death and senescence mechanisms, such genomic damage has been proposed to accelerate transformation and cancer progression.

Besides creating a stress response, however, ROS clearly participate in physiologic signaling at a variety of levels, requiring tight spatial and temporal regulation of oxidants by normal cells (Terada, 2006). In this capacity, ROS control proliferation, differentiation, junction formation, and response to cytokines and other soluble factors. Given their propensity to produce increased levels of ROS, are cancer cells able to appropriate these oxidant-dependent signals as a means of dysregulating proliferative and survival pathways? In this issue of *Cancer Cell*, Zhu et al. (2011) uncover a role for angiopoietin-like 4 (ANGPTL4) in activating integrin-related, oxidant-dependent survival pathways, despite the loss of matrix attachment. This highjacking of normal

anchorage-sensing mechanisms sheds further light on the complex role of ROS in malignant cell behavior.

ANGPTL4 has previously been shown to regulate lipid metabolism; interestingly, fasting and hypoxia induce ANGPTL4, suggesting its possible importance in the tumor microenvironment. Accordingly, upregulation of ANGPTL4 predicts metastasis of breast cancer to the lung, possibly by preparing the lung microvascular bed for efficient tumor cell extravasation (Padua et al., 2008). In the present study, Zhu et al. (2011) first survey ANGPTL4 expression in normal and neoplastic cells and tissue and find elevated protein and mRNA levels in adenomas and carcinomas from a variety of tissues besides breast, suggesting broad relevance in human cancers. Further, in knockdown or knockout studies of both human skin carcinoma xenograft and mouse melanoma tumor models, the authors show that ANGPTL4 supports tumor growth in an autocrine or paracrine fashion. Of importance, treatment with an antibody against ANGPTL4 significantly retards melanoma growth in the mouse model, indicating that this pathway may be clinically targetable.

The authors further studied the effects of ANGPTL4 on attachment sensation by tumor cells. Anchorage independence is a well-known mesenchymal phenotype that allows carcinomas to metastasize and possibly to expand as a mass without consistent basement membrane contact. The mechanisms by which normal epithelioid cells sense attachment and are committed to death following detachment are not well understood, but clearly involve outside-in integrin signals. Here, the authors demonstrate direct binding of ANGPTL4 to β_1 and β_5 integrins, resulting in the activation of both Rac1 and FAK and the downstream activation of Src, Akt/PKB, and ERK with consequent protection from anoikis. Thus, ANGPTL4 is capable of falsely reporting anchored conditions through integrin binding.

The composition of the integrin-related signaling complex that reports anchorage conditions is not well understood. Rac1 in particular is critical to coupling integrin ligation with cell cycle progression (Mettouchi et al., 2001). Among a number of Rac1 effectors, several members of the NADPH oxidase (Nox) family are of potential relevance in this regard. Increased ROS have been associated with both detachment from and attachment to matrix, and are thought to transduce either death or survival signals. In the latter instance, oxidative activation of Src has been shown to mediate survival effects (Giannoni et al., 2005), though the specific oxidant source has been less clear. Of note, the Nox adaptor $p47^{phox}$ translocates to focal complexes and initiates local Rac1 and Src-dependent redox signaling in migrating cells, indicating Nox signaling that is restricted to sites of nascent integrin clustering (Wu et al., 2005). This adaptor serves Nox1 as well as Nox2, which both require Rac1 for activation.

In the present article, the authors clearly demonstrate Nox1 as the predominant oxidase mediating ANGPTL4/$\beta_{1\text{-}2}$ integrin-dependent survival signals (Zhu et al., 2011). Identification of this particular oxidase is notable, as it was originally described as a mitogenic oxidase associated with cell transformation (Suh et al., 1999). Indeed, subsequent studies have linked Nox1 induction, downstream from oncogenic Ras, with anchorage independence and tumorigenesis (Mitsushita et al., 2004). More recently, Nox1 has also been shown to mediate β_1 integrin signaling during directional migration and wound repair (Jun and Lau, 2010; Sadok et al., 2009), suggesting a broader role for Nox1 in mediating β_1 integrin signaling. Together, these data suggest that Nox1 may exert its signaling effects primarily through its integration into specific integrin complexes.

This study has interesting implications and raises several general questions. First, the ability of ANGPTL4 to activate PI3K and ERK pathways suggests a common involvement of Ras, whose effects are known to be linked to integrin ligation context. While the authors did not specifically examine Ras involvement, Ras and Nox control broadly overlapping cellular functions, including proliferation, survival, cell shape change, and motility. Ras has been shown to be activated downstream of oxidants, either through direct cysteine modification or through upstream events; conversely, oncogenic Ras is known to exert mitogenic effects through ROS. In addition, both Ras and Nox gene families arose early in eukaryotic evolution, appear to collaborate in specific developmental and morphologic adaptations to environmental stress, and colocalize in relevant signaling compartments. Thus, it is conceivable that ANGPTL4 may feed into an evolutionarily conserved environment-sensing pathway.

One question raised is the relevance of the specific oxidant produced. While the authors correlate ANGPTL4 effects with changes in O_2-:H_2O_2 ratios, caution should be exerted with respect to the significance of this ratio. First, one rarely, if ever, encounters O_2- without H_2O_2, owing to its rapid spontaneous dismutation, making it often difficult to distinguish the effects of one oxidant species from the other in vivo. Second, despite their differing chemical reactivity, common molecular targets have been ascribed to both. For instance, Src oxidation during cell adhesion, as postulated in the present study, is blocked by PEG-catalase, indicating H_2O_2 and not O_2- as the proximal ROS (Giannoni et al., 2005). Third, it is probable that the observed changes in O_2-:H_2O_2 ratios reflect alterations in activity of specific oxidases induced by the various interventions. A shift in the activity or presence of various oxidases, which are differentially localized and regulated, is more likely to be responsible for changes in signal output. For example, Nox4, a predominantly H_2O_2-producing Nox, is localized to the ER and mediates ER stress signaling and cell fate decisions, and may in part be responsible for the increase in cell death.

Perhaps the most interesting question has to do with how ANGPTL4 can fool the cell to believe that it is anchored to a solid environment. Soluble matrix fragments or peptide-coated microspheres will ligate and cluster integrins but not prevent anoikis, suggesting that proper anchorage sensing requires a mechanical readout of the stiffness of the surrounding matrix, beyond simple integrin ligation. Such mechanosensation appears to require RhoA-dependent tension against the underlying solid matrix (Ma et al., 2007). In this regard, it is noteworthy that Nox1 locally inactivates RhoA (Sadok et al., 2009), offering a possible means to defeat this tension test. Further study of this integrin-related signaling complex will lead to deeper insight into the mechanism of anchorage sensing and its loss in cancer cells.

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SIRT3 Controls Cancer Metabolic Reprogramming by Regulating ROS and HIF

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In this issue of *Cancer Cell*, Finley and coworkers report that the genetic loss of the deacetylase SIRT3 leads to metabolic reprogramming toward glycolysis. This shift is mediated by an increase in cellular reactive oxygen species (ROS) generation that amplifies HIF-α stabilization and HIF-dependent gene expression, thereby driving the tumor phenotype.

In cancer cells, reprogramming of cellular metabolism drives substrate utilization toward a dependence on glucose. First described by Otto Warburg (Warburg, 1956), the significance of this response for tumor growth has been controversial. However, it appears that this glycolytic shift is necessary to provide a source of substrates for the synthesis of amino acid, lipids, and nucleic acids that are needed for proliferation (Vander Heiden et al., 2009). Indeed, enhanced glucose uptake by tumor cells forms the basis for the clinical detection of tumors by imaging regions exhibiting increased uptake of the glucose analog ^{18}F-fluordeoxyglucose. While the association between cancer and the Warburg metabolic shift is well established, the cellular mechanisms regulating this response are not fully understood.

Posttranslational modifications of proteins are important for regulating their function in health and disease. Critical roles for protein deacetylases are also emerging in cancer. For example, Kim et al. (2010) identified a role for SIRT3, a member of the seven-member sirtuin family, as a tumor suppressor. They showed that genetic deletion of SIRT3 pushes the cell in the direction of oncogenic transformation. While activation of two oncogenes (such as Myc and Ras) is needed to transform an immortalized fibroblast into a tumor-forming cell, genetic deletion of SIRT3 reduced that number to one. Thus, SIRT3 functions as a tumor suppressor (Schumacker, 2010). The mechanistic basis for SIRT3's tumor-suppressive role seems to reside in its ability to regulate reactive oxygen species (ROS) generation or clearance by the cell. Kim et al. (2010) noted that ROS levels were increased in SIRT3$^{-/-}$ cells, as a consequence of a decreased expression of antioxidant enzymes such as catalase and MnSOD. The transcription factor FOXO3a plays an important role in regulating the expression of MnSOD and other antioxidants, and SIRT3-mediated deacetylation of FOXO3a promotes its nuclear localization (Jacobs et al., 2008). Thus, the loss of SIRT3 activity suppresses FOXO3a, leading to an increase in cellular ROS signaling. Enhanced ROS levels have been linked to cancer, and Kim et al. (2010) observed an increase in the incidence of mammary tumors in the SIRT3 knockout mice. They suggested that the chronic increase in mitochondrial ROS stress might result in mitochondrial or genomic DNA damage, but that mechanism was not directly tested. Nevertheless, their study identified an important pathway by which SIRT3 suppresses tumor cell survival and proliferation through its effects on cellular ROS regulation.

But even the best studies leave many questions unanswered. The principal issue left in the wake of the Kim et al. (2010) study related to how the increase in ROS (caused by loss of SIRT3) mediates the enhanced tumor phenotype of cells. The answer to that question arrives in the article by Finley et al. (2011) in this