



by the observation that the most sensitive clinical test for detecting occult metastases is the uptake of [¹⁸F]fluorodeoxyglucose as determined by PET (18). Can a characteristic that is so useful for diagnosis also be exploited for therapy? Research that elucidated the mechanisms by which cancer cells induce blood vessel growth led to the development of angiogenesis inhibitors as anticancer agents over the last decade (19). Inhibitors of HIF-1, LDHA, PDK1, CA9, NHE1, and now MCT1 have shown anticancer effects in tumor xenograft models (11, 17, 20). It is likely that over the next decade, clinical translation of advances in our understanding of cancer metabolism will have an impact on cancer therapy. Furthermore, a treatment strategy in which tumors are deprived of both O₂ (through angiogenesis inhibition) and their ability to adapt to hypoxia (through metabolic inhibition) may represent a formidable combination therapy.

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Autophagy-induced tumor dormancy in ovarian cancer

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Autophagy — a process of “self-eating” that involves enzymatic digestion and recycling of cellular constituents in response to stress — contributes to both cancer cell death and survival. In this issue of the *JCI*, Lu et al. report that controlled induction of tumor suppressor gene aplasia Ras homolog member I (ARHI) results in autophagic cell death of human ovarian cancer cells in vitro (see the related article, doi:10.1172/JCI35512). However, within xenograft tumors in mice, multiple factors within the tumor microenvironment switched ARHI-induced autophagy to a mechanism of tumor cell survival, leading to tumor dormancy. Since ARHI expression is suppressed in the majority of breast and ovarian cancers but is high in premalignant lesions, ARHI-induced autophagy could be manipulated for therapeutic benefit.

Nonstandard abbreviations used: ARHI, aplasia Ras homolog member I; *BECN1*, beclin 1.

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Cells that encounter a variety of stresses undergo an evolutionarily conserved process of self-digestion termed *autophagy*. The importance of this intracellular damage response for pathophysiology has been established across multiple fields, includ-

ing infectious disease, neurodegeneration, heart failure, and cancer (1). In cancer in particular, the debate continues to rage as to whether or not autophagy is primarily a mechanism of cell death or cell survival. This is important to understand in order to preferentially promote the clinical development of therapeutic interventions that can either inhibit or enhance autophagy in tumor cells. A number of clinically available cancer therapeutics, including DNA-damaging chemotherapy, radiation therapy, and molecularly targeted therapies have been found to induce autophagy in cell culture and animal models (2). Recent investigations have found that inhibition of therapy-induced autophagy with chloroquine derivatives can enhance cell death in estab-

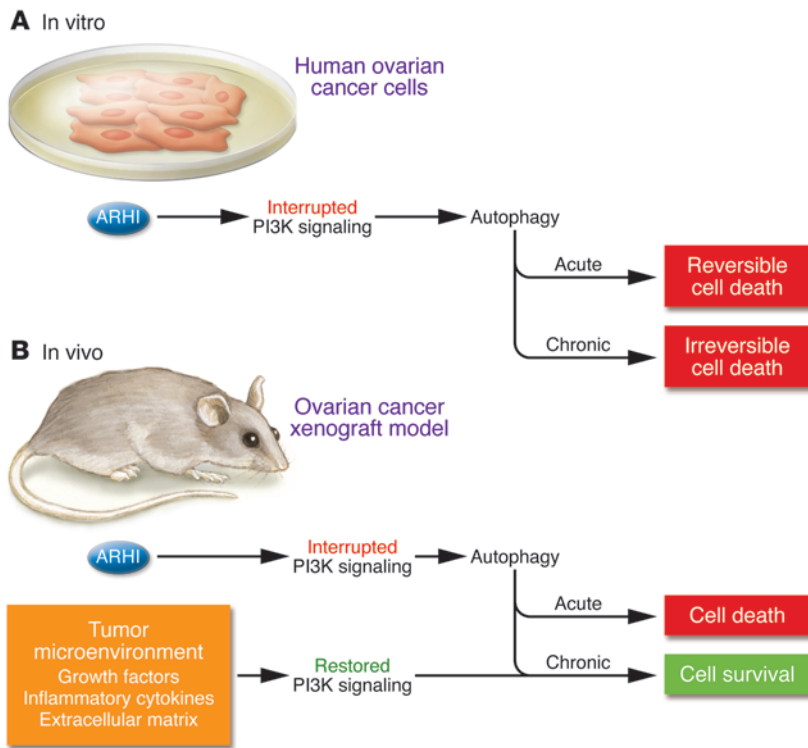


Figure 1

Consequences of forced expression of the tumor suppressor gene *ARHI* in ovarian cancer. In their study in this issue of the *JCI*, Lu et al. (8) report that controlled expression of the tumor suppressor gene *ARHI* in human ovarian cancer cells leads to autophagy. (A) *ARHI* expression was associated with interrupted PI3K signaling, which resulted in reversible (under conditions of transient *ARHI* expression) or irreversible (under conditions of chronic *ARHI* expression) autophagic cell death in vitro. (B) In contrast, *ARHI*-induced autophagy contributed to cell survival and tumor dormancy in ovarian cancer xenografts in mice, due to partially restored PI3K signaling from elements of the tumor microenvironment.

lished tumors, leading to enhanced tumor regression and delayed tumor regrowth (3). In this study, a combination of experiments involving RNA interference and drugs were used to demonstrate that therapy-induced autophagy in established tumors allows tumor cells to survive therapeutic stress (3). The cytoprotective role of autophagy following chemotherapy has been confirmed by other investigators (4–6). These findings have led to a growing number of early-phase clinical trials that aim to establish the safety and preliminary efficacy of combining agents that induce autophagy with hydroxychloroquine in patients with advanced malignancies refractory to standard treatments. Collectively, these actively accruing clinical trials represent the first attempt to manipulate autophagy for therapeutic benefit in cancer.

Autophagy inhibition with chloroquine has also been found to significantly delay the onset of spontaneous tumors in a transgenic mouse model of Myc-induced lymphoma (7). This study suggested that autophagy inhibition may have a role not only in combination therapies for patients with active metastatic disease, but also in the prevention of early disease. However, conducting a clinical trial of a novel autophagy inhibitor, or in the case of hydroxychloroquine, an old drug being

used for new indication, in tumor-free patients is difficult, requiring large numbers of patients, and long periods of follow-up. While the known safety and tolerability of chloroquine derivatives suggest they may be ideal drugs to test as chemopreventative agents, committing the resources necessary to conduct prevention trials may not be warranted until a potential benefit of this strategy has been established in a clinical setting with short-term outcomes, such as in metastatic disease. Another clinical setting that could be more feasible to investigate autophagy inhibitors is in the setting of minimal residual disease with high risk of relapse. A common problem across multiple malignancies is that cancer therapy can initially result in a complete remission, but there is an extremely high chance of relapse. This indicates there are clinically undetectable tumor cells that are dormant and have the ability to regrow at a later date. This is an ideal setting to investigate the role of autophagy and the effects of pharmacological autophagy inhibitors.

In this issue of the *JCI*, Lu, Bast, and colleagues report an ovarian cancer model of minimal residual disease and identify a role for autophagy in supporting tumor dormancy (8). Additionally, their analysis of the effects of controlled expression of the autophagy-inducing tumor suppressor

gene aplasia Ras homolog member I (*ARHI*) in human ovarian cancer cells in vitro versus in vivo sheds light on the relationship between autophagic cell death and autophagic cell survival and implicates an underappreciated role for the tumor microenvironment in deciding this fate.

The ras homolog *ARHI* activates autophagy in ovarian cancer

Over the past decade, Bast and colleagues have extensively characterized the genetic and biochemical underpinnings of *ARHI*, a maternally imprinted Ras-homologous kinase that exerts growth-inhibitory effects when expressed in ovarian cancer cells (9). *ARHI* is expressed in normal ovarian epithelium, but through the transition to malignancy, *ARHI* expression is suppressed in a stepwise fashion. Examination of tumor samples from cancer patients has determined that *ARHI* loss of heterozygosity (LOH) occurs in 60%–70% of ovarian and breast cancers (10–12). Another gene whose expression is suppressed through the LOH in breast and ovarian cancer is the autophagy-associated gene beclin 1 (*BECN1*). Unlike *BECN1*, in which the wild-type allele is retained and therefore can be expressed (13), the retained allele of *ARHI* is maternally imprinted and therefore not expressed (14). Since there appears to be

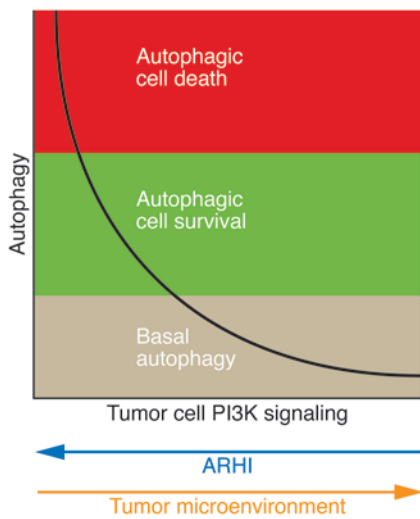


Figure 2

The magnitude of autophagy may determine the fate of cancer cells in vivo. This conceptual model proposes a relationship between the magnitude of cancer cell autophagy and its consequences on cell death and cell survival. As reported by Lu et al., expression of the tumor suppressor gene *ARHI* suppresses signal transduction through PI3K signaling, while factors within the tumor microenvironment maintain PI3K signaling within tumor cells (8). The magnitude of autophagy may be inversely proportional to PI3K signaling, and thresholds may exist within cancer cells for the magnitude of autophagy that leads to cell death versus the magnitude of autophagy that leads to cell survival.

a selective advantage to complete loss of *ARHI* expression during neoplastic transformation, developing a tumor model that allows inducible expression of *ARHI* at physiological levels in established tumors has provided valuable insight into the *ARHI*-mediated mechanism of tumor suppression. The insight gained from this current study by Lu et al. (8) has the potential to launch clinical investigations aimed at changing therapeutic approaches to the treatment of ovarian and breast cancers.

Using doxycycline-inducible expression of *ARHI*, the authors demonstrated that stable or transient physiologic expression of *ARHI* in human ovarian cancer cell lines and other human cancer cell lines resulted in the induction of autophagy and not apoptosis (8). Elegant in-cell cross-linking studies demonstrated that *ARHI* localizes with the autophagy marker light chain 3 (LC3) on the surface of autophagic vesicles, providing evidence that *ARHI* may regulate autophagy through direct physical

interaction with the autophagic machinery. Expression of *ARHI* was associated with increased expression of *ATG4*, which is encoded by one of the core autophagy genes (*ATG4*) in a group of genes denoted as *ATGs*. This oxidant-sensitive enzyme deconjugates LC3 from lipids on the surface of autophagic vesicles. To look for more indirect means by which *ARHI* could regulate autophagy, the authors used reverse-phase protein microarray analysis to look at multiple signaling events simultaneously. Expression of *ARHI* led to a marked inhibition of PI3K/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) and MAPK signaling. Expression of the mutant *ARHI* that was missing the N-terminal domain, which distinguishes its structure from other Ras family proteins, prevented growth factor-induced membrane recruitment of AKT and limited the activation of downstream effectors mTOR and p70S6K.

Death or survival from *ARHI*-induced autophagy

To investigate the functional consequences of *ARHI*-induced autophagy, the authors determined that *ARHI* expression prevented clonogenic growth after one week and resulted in cell death after two weeks (8). In contrast to a previous study in which chronic growth factor withdrawal from apoptosis-deficient cells resulted in a reversible form of autophagy that contributed to cell survival (15), Lu et al. show that transient expression of *ARHI* in human ovarian cancer cells leads to a reversible form of autophagy, but that chronic *ARHI* expression leads to irreversible autophagic cell death (Figure 1A). When *ARHI* expression was induced in ovarian cancer xenografts in mice, these authors found that acute cell death ensued, leading to tumor regression. However, removal of doxycycline, which resulted in the absence of *ARHI* expression, led to tumor regrowth. These dormant cells were likely dependant on autophagy as a survival strategy, since systemic pretreatment of mice harboring *ARHI*-suppressed ovarian xenografts with the autophagy inhibitor chloroquine resulted in a significant delay of tumor regrowth once *ARHI* expression was downregulated.

These results suggest that, unlike the in vitro experiments, in which cells exposed to *ARHI* were destined for autophagic cell death, within the tumor microenvironment, *ARHI*-induced autophagy allows a cohort of hibernating cells to survive and

eventually recur as tumors once the *ARHI* tumor suppressor is downregulated (8). Lu et al. investigated the contributions of growth factors, inflammatory cytokines, angiogenic factors, and extracellular matrix proteins to autophagic cell death in vitro and in vivo. They found that, by limiting the *ARHI*-dependent suppression of intracellular PI3K signaling, members of each of these classes of molecules within the tumor microenvironment can contribute to changing autophagy from a cell death mechanism to a cell survival mechanism. Taken together, these results suggest that the magnitude of autophagy is inversely related to PI3K signaling. *ARHI* suppresses intracellular PI3K signaling, and elements of the tumor microenvironment promote intracellular PI3K signaling. Autophagic cell death occurs when autophagy is maximally induced, but when levels of autophagy are elevated above basal levels, but are less than maximum levels, the result is autophagic cell survival (Figure 2).

Tumor suppressor genes and autophagy

This microenvironment-directed switch may also shed light on why a gene whose expression can suppress multiple signaling pathways and lead to cell death is expressed constitutively in normal and premalignant epithelium. The role of autophagy in limiting DNA damage and genomic instability has been established (16). One hypothesis that can be tested in future studies is that *ARHI*-induced autophagy limits the rate of genomic instability and neoplastic transformation, while microenvironmental signals in normal or premalignant native tissue simultaneously limit *ARHI*-induced autophagic cell death. A related question that arises is whether the effects of *ARHI* expression are specific to ovarian and breast cancer, or whether *ARHI* expression is selectively suppressed in other cancers. Similarly, what is the interaction between *ARHI* expression and common mutations in oncogenes encountered in ovarian cancer? The SKOV3 ovarian cancer cell line has constitutively activated PI3K/AKT signaling but no known mutations in *PI3K*, phosphatase and tensin homolog (*PTEN*), *RAS*, or *BRAF*. Would the effects of *ARHI* expression be different in tumor cells harboring mutations in *PI3K*, *RAS*, or *BRAF*? Finally, *ARHI* can be added to a growing list of “non-*ATG*” genes that were not originally identified as autophagy genes but that directly regulate this pro-



cess through physical engagement of the autophagic machinery. This list includes, but is not limited to, endophilin B1 (*BIF1*) (17), UV radiation resistance-associated gene (*UVRAG*) (17), *p62* (18), and WD repeat domain phosphoinositide-interacting 1 (*WIPI1*) (19). Individually, aberrant expression of some of these genes has been demonstrated in a wide variety of malignancies. It remains to be determined whether more or all of these genes are bona fide tumor suppressor genes in human tumors. It will also be important to determine whether the genetic aberrations in multiple autophagy-associated genes can be found in the same tumor, as well as the implications of this for tumorigenesis and clinical prognosis.

Clinical implications of the inducible-ARHI tumor model

Regardless of these remaining questions, the inducible ARHI tumor model reported here (8) also has practical implications in understanding the role of autophagy in the treatment of ovarian cancer patients. The authors point out that 60% of primary ovarian tumors have decreased ARHI expression associated with shortened disease-free survival after chemotherapy. This observation could be explained by a propensity for early recurrence in ovarian tumors lacking ARHI-induced autophagy, which suggests that disease-free survival could be prolonged in this subgroup of patients by combining drugs known to induce autophagy with current chemotherapy regimens. Alternatively, in patients whose tumors express ARHI, could the addition to standard chemotherapy of an autophagy inhibitor prolong disease-free survival compared with chemotherapy alone? Examining the tumor tissue of

ARHI-positive and ARHI-negative tumors for markers of autophagy is a first step toward determining whether these clinical investigations should be launched. A parallel preclinical effort would be to exploit this tumor model as a platform to validate autophagy inhibitors with pharmacological properties identified in drug screens. Promising autophagy inhibitors should reproducibly delay or prevent regrowth of ARHI-suppressed tumors upon removal of doxycycline. While most tumor suppressor genes are not druggable targets themselves, the current study by Lu et al. (8) demonstrates that mechanistic studies of tumor suppressor genes can uncover druggable targets that can be tested in defined subsets of cancer patients.

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