RAS-mediated tumor stress adaptation and the targeting opportunities it presents

Alexandra Redding, Andrew E. Aplin and Elda Grabocka*

ABSTRACT
Cellular stress is known to function in synergistic cooperation with oncogenic mutations during tumorigenesis to drive cancer progression. Oncogenic RAS is a strong inducer of a variety of pro-tumorigenic cellular stresses, and also enhances the ability of cells to tolerate these stresses through multiple mechanisms. Many of these oncogenic, RAS-driven, stress-adaptive mechanisms have also been implicated in tolerance and resistance to chemotherapy and to therapies that target the RAS pathway. Understanding how oncogenic RAS shapes cellular stress adaptation and how this functions in drug resistance is of vital importance for identifying new therapeutic targets and therapeutic combinations to treat RAS-driven cancers.

KEY WORDS: RAS, Tumor-associated stress, RAS-pathway targeting, Drug resistance, Stress adaptation

INTRODUCTION
The RAS pathway responds to external growth factors by activating genes that regulate several biological processes, including cell growth, division and differentiation. The pathway begins with the binding of growth factors to their cognate receptor at the cell surface, leading to the activation of the three isoforms of the small GTPase RAS (HRAS, KRAS and NRAS). RAS activation initiates multiple signaling cascades, which culminate in the activation of transcription factors, such as c-Myc (also known as MYC), c-JUN (also known as JUN), and ETS and CREB proteins (Chang et al., 2003). The hyperactivation of the RAS pathway due to the acquisition of activating mutations in RAS is an initiating event in malignant transformation; ~19% of all cancer patients harbor an activating mutation in one of the RAS genes (Prior et al., 2020). As such, this prevalent oncogenic driver presents an opportune target in the treatment of a variety of cancer subtypes. However, inhibiting the RAS protein in a clinical context has proven challenging for a variety of reasons (Choi et al., 2019). These include its active site distribution and reproduction in any medium provided that the original work is properly attributed.
RAS-driven tumors to better understand how oncogenic RAS operates as a primary inducer of stress, and in response to stress, to favor survival. These stress-adaptive mechanisms are pertinent to understanding therapeutic outcome in the clinic, as resistance is still a major setback when treating RAS-driven cancer. As new RAS pathway-targeting therapies arise, the investigation of therapy-induced stress adaptive pathways should be of great importance, as this may pinpoint appropriate targets that confer resistance for that specific therapy or cancer type.

**RAS-driven adaptations to cellular stress**

Oncogenic RAS directly induces various kinds of cellular stress, and these stress-adaptive responses have been implicated in promoting tumorigenesis. However, because survival in the face of stress often relies on the duration and severity of such stress, oncogenic RAS also upregulates pathways that aid in stress mitigation. This section will describe how oncogenic RAS induces stress-adaptive tumor-promoting pathways and keeps them in check by upregulating other pathways that modulate the stress intensity.

**Adaptation to oxidative stress**

Oxidative stress is defined as an imbalance in the levels of free radicals, and the inability to detoxify free radicals and their harmful effects. Heightened oxidative stress is a key feature of oncogenic RAS-driven cancers (Irani et al., 1997; Vafa et al., 2002). The increased formation of radical oxygen species (ROS), such as superoxide anion \((O_2^-)\) and hydrogen peroxide \((H_2O_2)\), is a common characteristic of cancer cells (Sztawrowski and Nathan, 1991). ROS are generated via the electron transport chain, via the activation of NADPH oxidases (such as NOX1), or through the activity of lipoxygenases, among other mechanisms. As they are major modulators of cell signaling and gene expression, certain levels of ROS are necessary for cellular function (Chandel et al., 2000; West et al., 2011). However, ROS are also damaging agents that can interact with DNA and proteins, cause lipid peroxidation and lead to apoptosis (Wang et al., 2008). Oxidative stress is kept in check by the antioxidant program – an intrinsic mechanism by which cells maintain an appropriate level of free radicals that opposes the formation and activity of ROS. Antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase and others, break down ROS into non-damaging molecules (Ighodaro and Akinloye, 2018). Scavenger molecules also readily react with ROS, reducing the interaction between ROS and cellular proteins through competition.

Oncogenic RAS increases ROS levels through a variety of mechanisms, including enhanced activity of NOX proteins, which produce superoxide. Specifically, RAS elevates ROS levels through MAPK-mediated transcriptional upregulation of Nox1 (Mitsushita et al., 2004), p38 (also known as MAPK11)-mediated stabilization and translocation of the p47phox subunit of Nox1 (Park et al., 2014), and via COX-2 (also known as PTGS2)-mediated prostaglandin E production, which generates \(H_2O_2\) as a byproduct (Maciag et al., 2004). In oncogenic RAS-driven human pancreatic tumors and in pancreatic cancer mouse models, the levels of the ROS-inducing NOX4 correlate positively with tumor progression, indicating a heightened reliance on ROS during tumorigenesis (Ogrunc et al., 2014). Oncogenic RAS and mutant BRAF also contribute to the antioxidant program, as their expression increases the transcriptional levels of Nrf2 (also known as NFE2L2), a transcription factor that binds to antioxidant response elements and promotes the expression of antioxidant genes (Demicola et al., 2011; Mukhopadhyay et al., 2020). This stress-adaptive mechanism is pro-tumorigenic, as Nr2−/− mouse models of pancreatic cancer have fewer pancreatic intraepithelial neoplasia (PanINs), which are also less proliferative and have higher levels of senescence compared to Nrf2-expressing counterparts (Demicola et al., 2011). The reduction in proliferation of Nrf2-deficient PanINs can be rescued by the addition of the antioxidant N-acetyl cysteine, indicating that this oncogenic RAS-driven antioxidant program contributes to PanIN formation and progression. In addition to supporting the antioxidant program, oncogenic RAS inhibits \(H_2O_2\)-induced apoptosis (Young et al., 2004). Together, these studies indicate that oncogenic RAS directly aids in the adaptation to the oxidative stress it induces, resulting in increased tumorigenesis in vivo.

**Adaptation to metabolic stress**

Cancer cells have a higher demand for nutrients and energy relative to non-transformed cells, to support their rapid levels of growth and proliferation, and often become stressed in the attempt to satisfy these metabolic needs. As a cancer cell population grows, it faces
overexpression of oncogenic KRASG11D and KRASG12D in human non-cancerous colon cells increases levels of autophagy, suggesting that mutant KRAS plays a specific role in autophagy induction under stress (Alves et al., 2015). The suppression of autophagy in KRASG12V-containing colorectal cancer patient-derived SW480 cells through the knockdown of ATG5 and BECN1, which are involved in autophagosome formation, increases cell death during starvation, highlighting the importance of autophagy in nutrient stress adaptation (Alves et al., 2015).

A third mechanism by which oncogenic KRAS combats nutrient deprivation is through its effect on the expression levels of GOT1 and GLUD1, two major enzymes involved in glutamine metabolism (Son et al., 2013). Pancreatic cancer cells use GOT1 to fuel the citric acid cycle while maintaining the redox state of the cell (Son et al., 2013). KRAS knockdown in multiple PDAC cell lines increases the mRNA and protein levels of GLUD1 and decreases GOT1 (Son et al., 2013). This effect was mimicked in vivo, as Got1 mRNA levels increased and Glud1 mRNA levels decreased with the induction of KRAS expression in a pancreas-specific doxycycline-activated oncogenic KRAS-inducible mouse model. GOT1 knockdown led to an approximate sixfold reduction in tumor volume in this model, indicating that GOT1 aids in tumor growth (Son et al., 2013).

Oncogenic RAS also activates a selective mitophagy program that reduces mitochondrial ROS specifically, and redirects glucose metabolism away from the mitochondria through the increased expression of BNIP3L (also known as NIX) (Humpton et al., 2019). BNIP3L is a pro-apoptotic Bcl-2 family member, the interaction of which at the mitochondrial outer membrane promotes the entry of lysosomal proteins from the cytoplasm into the mitochondrial matrix, leading to mitophagy. Mitophagy involves the degradation of mitochondria through autophagy, often as a result of cellular stress or damaged mitochondria or, as in this instance, as an output of oncogenic signaling. RAS-driven mitophagy leads to a reduction in mitochondrial content. It also leads to a BNIP3L-dependent decrease in mitochondrial glucose flux and citric acid cycle intermediates, changes that indicate a channeling of glucose into aerobic glycolysis or into other anabolic processes (Humpton et al., 2019). This altered metabolism was hypothesized to lead to an increased survival advantage for RAS-driven tumors, as BNIP3L depletion via siRNA reduced the proliferation of KRASG12D-expressing murine embryonic fibroblasts. In support of this, the conditional deletion of Bnip3 in pancreata of KC (KRASG12D-expressing) and KPC (KRASG12D and p53R172H-expressing) mouse models resulted in lower-grade PanINs, as well as an increase in median survival (Humpton et al., 2019). Therefore, RAS not only induces metabolic stress, but also contributes to multiple mechanisms that promote survival during metabolic stress.

Adaptation to ER stress
ER stress refers to an increased presence of unfolded proteins within the ER, which can arise from a variety of cancer-related insults, including hypoxia, oxidative stress, genomic instability, and enhanced protein production and secretion (Yang et al., 2014; Oakes, 2020). ER stress leads to the activation of the unfolded protein response (UPR), which can be both cytoprotective and cytotoxic, depending upon how well it can mitigate the accumulation of unfolded proteins. The UPR contains three main signaling nodes: inositol-requiring protein 1 (IRE1); also known as ERN1, activating transcription factor (ATF)-6, and PKR-like endoplasmic reticulum kinase (PERK; also known as EIF2AK3) (Yadav et al., 2014). Upon accumulation of misfolded proteins,
these ER transmembrane proteins respond by activating signaling cascades that promote transcriptional and translational changes, such as the transient re-localization of specific classes of mRNAs from the ER into the cytoplasm (Reid et al., 2014), which integrate to favor either a return to homeostasis or the induction of apoptosis (Kadowaki and Nishitoh, 2013; Acosta-Alvear et al., 2007; Hetz et al., 2006).

The UPR plays a significant role in cancer, as markers of this process are increased or altered across several cancer types (Fig. 2) (Yadav et al., 2014). In addition, the genetic ablation of Ire1a in intestinal epithelia-specific Ire1a-knockout mice (Li et al., 2017) and the mammary gland-specific knockout of Perk in mammary tumor-prone MMTV-Neu mice (Bobrovnikova-Marjon et al., 2010) reduce cancer growth and initiation, respectively, and the expression of PERK has been linked to chemo-resistance in colon cancer cells and in subcutaneous xenograft models of colon cancer in NOD/SCID mice (Shi et al., 2019). The UPR pathway is upregulated in a variety of RAS mutant cancers and along the axis of RAS-driven tumor progression (Blazanin et al., 2017; Denoyelle et al., 2006; Catanzaro et al., 2014). In human and murine tissue samples of oncogenic RAS-driven acinar-to-ductal metaplasia (ADM) and PDAC, the ER stress-sensing protein GRP78 (also known as HSPA5) is upregulated in ADM and PDAC lesions, whereas little to no GRP78 is detected in corresponding wild-type samples (Hill et al., 2012). GRP78 disassociates from the ER transmembrane proteins IRE1, PERK and ATF6 under ER stress, leading to their dimerization and activation, which promotes UPR. In terms of tumorigenicity, GRP78 also contributes to various stem-like properties of pancreatic cancer cells, such as clonogenicity, self-renewal and invasion, which translate into a reduced capacity to initiate tumor formation and to decreased tumor weight in nude mice subcutaneously injected with pancreatic cancer cells (Dauer et al., 2019). Although this evidence supports the pro-tumorigenic properties of ER stress, it is important to note that it can also be anti-tumorigenic based upon severity and duration, which can explain the dual nature of oncogenic RAS in inducing or limiting the UPR (Maurel et al., 2015).

Consistent with the pro-tumorigenic role of ER stress, oncogenic RAS can directly impact ER stress levels through the activation of IRE1a via the MEK–ERK pathway (Blazanin et al., 2017). The expression of oncogenic HRAS in primary murine keratinocytes increases Ire1a mRNA and protein levels and its phosphorylation, indicative of the overall Ire1a activation (Blazanin et al., 2017). In addition, oncogenic RAS induces ER stress indirectly through ROS

---

**Fig. 2. Oncogenic RAS-driven induction of stress-adaptive mechanisms and current therapies along the signaling axis.** Oncogenic RAS induces multiple stress-adaptive pathways, such as altered (glucose) metabolism, UPR, DDR, autophagy, macropinocytosis and stress granule formation. Canonical oncogenic RAS signaling, such as the activation of the MEK–ERK1/2 and PI3K–AKT pathways, which directly promote proliferation, is also displayed. There are multiple drugs in clinical trials that target different nodes within these stress-adaptive and canonical pathways, as shown in red. There are still mutant RAS-driven stress-adaptive pathways that have yet to be targeted in the clinic, such as the formation of stress granules. CQ, chloroquine; DDR, DNA damage response; HCQ, hydroxychloroquine; OxPhos, oxidative phosphorylation; TCA, tricarboxylic acid cycle; UPR, unfolded protein response.
induction (Park et al., 2014; Mitsushita et al., 2004). As discussed above, ER stress can result in both cell survival and cell death, and tumorigenic progression requires the tempering of and/or adaptation to ER stress. IRE1α activation by oncogenic RAS results in the splicing of X-box-binding protein 1 (Xbp1), which has been implicated in stress adaptation during the UPR (Blazanin et al., 2017; Hollien et al., 2009). ER stress favors this particular activity of IRE1α, which was shown to be necessary for mutant HRAS-expressing cells to proliferate. By contrast, reduced ER stress favors senescence despite the retention of activated IRE1α. This shows that both the presence of ER stress and the activation of IRE1α through oncogenic RAS work together to promote a proliferation-supportive phenotype. Oncogenic RAS also specifically upregulates proteins that limit the ER stress response. In patient-derived myeloma cell lines that were engineered to constitutively express mutant forms of either KRAS, NRAS or BRAF, the expression of each of these oncogenes increased the transcription of proteasome 20S subunit beta 8, 9 and 10 (PSMB8, PSMB9 and PSMB10) (Shirazi et al., 2020). The transcriptional levels of the assembly chaperone proteasome maturation protein (POMP) and its upstream regulator Nrf2, which are required for the cleavage and activation of PSMB8/9/10, were also increased after the expression of the KRAS, NRAS or BRAF oncogenes (Shirazi et al., 2020). These results suggest that oncogenic RAS and BRAF may enhance proteasome capacity, which could mitigate the activation of the ER stress response through a reduction in proteotoxic stress. Surprisingly, the expression of these oncogenes also reduced the transcription of ATF4 and ATF6, which are involved in the ER stress response, showing that oncogenic RAS can directly dampen ER stress signaling as well.

Adaptation to hypoxia

Hypoxia describes a state of low or inadequate oxygen availability, and can exist at the cell, tissue or organ level (Muz et al., 2015). It often occurs as a result of reduced blood flow to a particular region or because of the increased proliferation of cells within a tissue, such as in a tumor, where highly proliferative cancer cells consume more oxygen than normal cells and eventually outgrow their initial supply. Cells undergoing hypoxia respond by stabilizing hypoxia inducible factor 1 subunit alpha (HIF-1α), a transcription factor responsible for the activation of multiple genes involved in metabolism and angiogenesis, and vascular endothelial growth factor (VEGF; also known as VEGFA), which promotes angiogenesis to increase blood supply (Forsythe et al., 1996). Cancer cells are notorious for generating a dysfunctional vasculature through their stimulation of angiogenesis. Different isoforms of VEGF exist, and their activation can lead to differential vascularization patterns within a tumor (Yu et al., 2002). As this vascularization changes, the distinct spatial regions of a tumor experience periods of hypoxia and normoxia, leading to environmental pressures that select for cells that can survive under such conditions. In addition, hypoxia can induce epithelial-to-mesenchymal transition, which enhances the invasive and metastatic properties of cancer cells (Muz et al., 2015). Different organs across the body have varying levels of oxygen that are considered physiological, and each of these tissues experiences a specific drop in oxygen levels when a tumor is present (Muz et al., 2015). Therefore, in addition to the size of a tumor, the specific tissue it forms in can affect the extent of hypoxia.

Oncogenic RAS-driven tumors experience hypoxic conditions for the reasons described above, as oncogenic RAS increases cell proliferation. There are also a variety of mechanisms that link oncogenic RAS to the stress-adaptive mechanisms involved in cell survival during hypoxia. For example, the expression of oncogenic KRAS enhances HIF-1α function, and that of oncogenic BRAF enhances HIF-1α and HIF-2α (also known as EPAS1) function during hypoxia (Kikuchi et al., 2009). Receptor for advanced glycation end products (RAGE; also known as AGER), a protein primarily involved in inflammation, acts as a positive regulator of HIF-1α through its binding to oncogenic RAS during hypoxia (Kang et al., 2014). This binding is increased in human pancreatic cancer cells that express oncogenic RAS compared to a pancreatic cancer cell line that expresses wild-type RAS, suggesting that the mutational status of RAS may play a role in such binding (Kang et al., 2014). When MEK1/2 and AKT are inhibited in a murine pancreatic tumor cell line, RAGE can no longer activate HIF-1α, indicating that RAGE activates oncogenic RAS signaling to promote adaptation to hypoxic conditions (Kang et al., 2014). Moreover, knocking down RAGE in murine pancreatic tumor cell lines under hypoxia and knocking it out in KC mice in vivo reduces phospho-AKT and phospho-ERK1/2 levels (Kang et al., 2014). In addition to the activation of HIF-1α, oncogenic RAS has been shown to converge on a hypoxia-induced, stress-adaptive pathway that targets the tumor suppressor reversion-inducing cysteine-rich protein with Kazal motifs (RECK). RECK is a glycoprotein that downregulates matrix metalloproteinases that degrade extracellular matrix (ECM) proteins and contribute to tumorigenesis. RECK is inhibited during hypoxia through the activation of HIF-1α and miR-372/373 (Loayza-Puch et al., 2010). Oncogenic RAS contributes to this RECK inhibition through the upregulation of miR-21, potentially strengthening this response or priming the cell for survival during hypoxia (Loayza-Puch et al., 2010). Therefore, oncogenic RAS equips the cell to deal with a hypoxic environment, most notably by stabilizing HIF-1α and by converging on stress-adaptive pathways that inhibit the tumor suppressor RECK.

Adaptation to biomechanical stress

In order to survive, cells must be able to physically sense their microenvironment and to adapt to changes or respond to signals within that environment. There are a multitude of biomechanical sensing molecules that integrate these external signals into cellular responses, including cytoskeletal proteins, adhesion receptors and ion channels (Daniel et al., 2013; Yao et al., 2014; Lim et al., 2018). These sensing mechanisms can control cell shape, stiffness, motility, proliferation, survival and fate in response to what they sense within the surrounding environment. Changes in the biomechanical sensing mechanisms of cells, as well as changes in tension and homeostasis within a tissue overall, can be initiating events in tumorigenesis (Fernández-Sánchez et al., 2015; Razzaghi et al., 2012; Beverly et al., 2005; Pan et al., 2020).

Oncogenic RAS plays a role in the biomechanical properties of cells, and assists with cell survival in a physically changing microenvironment, such as during mitotic rounding and in responses that involve cellular stiffness (Matthews et al., 2020; Lin et al., 2015). For example, the expression of oncogenic HRAS in Madin-Darby canine kidney-derived epithelial cells and in mouse mammary gland epithelial cells, and the overexpression of oncogenic KRAS in human pancreatic ductal cells, result in cell softening compared to parental cell lines in vitro (Lin et al., 2015). In addition, the proliferative capacity of cancer cell lines with oncogenic KRAS was less affected than that of normal cells when challenged with soft matrix growth conditions, suggesting that oncogenic RAS can promote adaptation to biomechanical stress by modulating cell stiffness (Lin et al., 2015).
Oncogenic RAS has also been shown to directly affect the composition of the microenvironment, aiding in both cancer cell survival and metastasis. Transformation with oncogenic RAS leads to the overexpression of tenasin-C, an ECM molecule that can drive cancer progression (Maschler et al., 2004; Sun et al., 2018, 2019). Oncogenic RAS also promotes survival during ECM detachment (Mason et al., 2016). ECM detachment induces metabolic stress and the cell death program, anoikis, in normal cells, but is an initiating step in the metastasis of cancer cells (Mason et al., 2016; Schafer et al., 2009). When oncogenic RAS-expressing cells undergo ECM detachment, RAS blocks anoikis via the activation of serum/glucocorticoid-regulated kinase 1 (SGK-1) and the downregulation of PH domain leucine-rich repeat protein phosphatase (PHLPP; also known as PHLPP1), which inhibits the activation of the p38 MAPK pathway and blocks its role in anoikis, thus promoting survival and supporting metastasis (Mason et al., 2016). Therefore, oncogenic RAS mediates the stiffness-sensing mechanisms of the cell, affects the matrix of the surrounding microenvironment and favors cell survival during metastasis.

**Adaptation to pan-stress stimuli**

As described thus far, RAS-driven cancer cells are exposed to a range of cellular stresses, and oncogenic RAS can respond to these stresses in different ways by upregulating specific, stress-adaptive mechanisms. Oncogenic RAS can also respond to multiple stresses to enhance the overall stress tolerance of a cell, and these mechanisms can be considered adaptations to pan-stress stimuli. One of the major oncogenic, RAS-driven, pan-stress adaptations is the upregulation of stress granules (SGs) (Fig. 2). SGs are non-membranous cytoplasmic organelles that consist of protein and RNA and that assemble in response to various stress stimuli, such as hypoxia (Arimoto et al., 2008; Gottschald et al., 2010), oxidative stress (Namkoong et al., 2018), DNA damage (Byrd et al., 2016; Moutaoufik et al., 2014) and ER stress (Namkoong et al., 2018). SGs confer cytoprotection and promote survival, as evidenced by the fact that blocking SG formation under stress reduces cell survival in human breast and colon cancer cells in vitro (Arimoto et al., 2008; Grabocka and Bar-Sagi, 2016). SGs can directly oppose apoptosis by reducing ROS levels, through the sequestration of mammalian target of rapamycin complex 1 (mTORC1) via the spindle-associated protein atrasin, and through the sequestration of RACK1, a scaffolding protein involved in the stress-activated MAPK-driven apoptotic response (Arimoto et al., 2008; Takahashi et al., 2013; Thiedieck et al., 2013). Proteins that modulate SG assembly are upregulated in many human cancer types, including cancer, colorectal and prostate cancer, and sarcoma, and their expression levels often correlate with a poorer prognosis in the patient (Somasekharan et al., 2015; Sim et al., 2019; Li et al., 2020; Wang et al., 2021). *In vivo*, SGs have been implicated in metastasis, as osteosarcoma cells with knockdown of G3BP1 were associated with reduced levels of lung metastases upon implantation in the kidney capsule, compared to control osteosarcoma cells, which formed lung metastases within 4-5 weeks of implantation (Somasekharan et al., 2015).

Gain- and loss-of-function experiments in pancreatic and colorectal cancer cell lines demonstrate that oncogenic KRAS promotes SG formation as an adaptive mechanism to a variety of tumor-associated stress stimuli (Grabocka and Bar-Sagi, 2016). The induced expression of mutant HRAS also increases the SG-forming capacity of cells, suggesting that this phenotype may translate across mutant RAS isoforms (Grabocka and Bar-Sagi, 2016). Oncogenic KRAS-mediated SG assembly depends on the production of the lipid-signaling molecule 15-d-PGJ2, which occurs via the RAS–ERK-mediated regulation of two key enzymes, COX-2 and 15-hydroxyprostaglandin dehydrogenase (HPGD) (Grabocka and Bar-Sagi, 2016; Qiang et al., 2019). A particularly interesting aspect of this oncogenic RAS-induced stress response is that it can occur in a cell non-autonomous manner via the secretion of 15-d-PGJ2 (Grabocka and Bar-Sagi, 2016). Therefore, not only does oncogenic RAS enhance stress tolerance in the cell in which it operates, but it might also enhance the fitness of the surrounding cells in the microenvironment (Grabocka and Bar-Sagi, 2016). SGs might thus be a powerful RAS-induced stress-adaptive mechanism, as they are a singular output that responds to multiple challenges that a RAS-transformed cell faces. There is more to be uncovered about how SGs function in the different stages of tumorigenesis and about the specific mechanisms by which these granules combat the different stresses. Deriving answers to these questions would constitute an important step forward for the field, as such knowledge might aid in the identification of therapeutic targets that could hinder this pan-stress adaptation mechanism.

**Stress adaptation in the persister cell phenotype**

It is clear that multiple stress-adaptive pathways activated by oncogenic RAS can promote survival in the face of transformation-related stress. However, the way in which oncogenic RAS prepares the cell to deal with external stress, such as from the tumor microenvironment or chemotherapy, is also of great importance. One of these RAS-driven stress-adaptive pathways has recently been implicated in generating a stress-tolerant cell state, called a cycling persister cell. Tolerant cells are described as cells that have a reduced sensitivity to a particular drug or stress, whereas ‘persisters’ are cells that can enter into a dormant state to survive a particular drug or stress (Sharma et al., 2010; Kurppa et al., 2020). Although most persister cells remain dormant throughout a treatment, some can re-enter the cell cycle during treatment, and thus pose an immediate threat to a positive therapeutic outcome (Oren et al., 2021). Cellular programs that contribute to a cycling, persister cell phenotype during treatment have been described. The antioxidant program’s genes have been shown to be more highly expressed in cycling persister cell clones and to be targets of Nrf2 (Oren et al., 2021). When ROS levels are reduced in persister cells, through treatment with the scavenger molecule NAC or via the overexpression of the antioxidant enzyme glutathione peroxidase 2, the fraction of cycling persister cells increases by sixfold and threefold, respectively. This finding indicates that the activation of the antioxidant program might support the re-entry of persister cell populations into the cell cycle (Oren et al., 2021). Interestingly, oncogenic RAS increases ROS and Nrf2 expression levels, raising the possibility that RAS might contribute to the cycling persister cell population through this mechanism. The role of oncogenic RAS in promoting the emergence of persister cells and the ability of these cells to re-enter the cell cycle are important questions for future research to address.

Overall, oncogenic RAS is apt at providing survival mechanisms to combat its own stress induction, and may also contribute to an overall stress-tolerant phenotype that promotes endurance in the face of external stresses, such as chemotherapy. The following sections will describe how several resistance mechanisms are borne out of these oncogenic RAS-driven, stress-adaptive pathways, suggesting that oncogenic RAS also functions on the axis of external stress and chemoresistance.
Stress adaptation in drug tolerance and tumor resistance

Unbiased drug screens for synthetic lethality and for other multifaceted vulnerabilities of mutant RAS-driven cancer cells have identified specific stress response proteins and entire stress-adaptive pathways that, when inactivated, lead to increased cell death, decreased tumorigenesis and decreased tumor progression (Yang et al., 2019; Elliott et al., 2019; Luo et al., 2009). Findings from these screens support the idea that stress-adaptive responses are key contributors to the survival and resistance mechanisms of RAS mutant cancer cells, and they provide evidence that the targeting of these pathways can overcome resistance to the targeting of RAS itself and of RAS pathway components. In this section, we describe how the inhibition of stress-adaptive pathways might challenge some of the current clinical problems concerning resistance and we consider how therapy itself might induce particular stresses that lead to novel stress-response vulnerabilities in these tumors. Of note, oncogenic RAS cells also utilize adaptive mechanisms to promote resistance to conventional chemotherapeutics, which rely largely on DNA damage. The role of oncogenic RAS in inducing the DNA damage response (DDR), in promoting adaptation to DDR (Fig. 2), and the therapeutic strategies for targeting this stress response have been reviewed in detail elsewhere and are summarized in Table 1 (Grabocka et al., 2015; Reid et al., 2014; Li et al., 2021b). Some characteristics of RAS mutant cells that are pertinent to the response to classic chemotherapy and the reported resistance mechanisms that utilize the cellular stress response are summarized in Table 2.

Autophagy in drug response

The survival and progression of RAS mutant tumors has a complex relationship with autophagy, and the dependence of these tumors on autophagy has been well documented (Fig. 2) (Guo et al., 2011; Poillet-Perez et al., 2015; Guo et al., 2016; Lock et al., 2014). Autophagy-related 7 (ATG7) regulates autophagosome formation and is required for autophagy to occur. In an oncogenic KRAS-driven non-small cell lung cancer (NSCLC) mouse model, deleting Atg7 specifically in tumor cells reduced the tumor burden compared to that in mice with Atg7-expressing NSCLC. In addition, these Atg7-deficient adenomas progressed into oncocytomas as opposed to the adenocarcinomas seen in Atg7-expressing mice (Guo et al., 2013). An attractive clinical strategy has been to inhibit autophagy in such tumors; however, these monotherapies have ultimately failed due to sustained disease progression (Wolpin et al., 2014). More recently, data supporting the combined inhibition of autophagy and various proteins in the oncogenic RAS pathway has brought autophagy back into the spotlight (Bryant et al., 2019; Kinsey et al., 2019; Leung et al., 2018; Lee et al., 2019). For example, a recently identified small molecule, deltarasin, can disrupt the association of the chaperone phosphodiesterase-δ (PDEδ) with RAS, preventing PDEδ-mediated recruitment of RAS to the plasma membrane and therefore its activation (Leung et al., 2018). Deltarasin alone has a strong impact on tumor weight in a lung cancer cell xenograft mouse model; the average tumor weight of deltarasin-treated mice was 57% less than that of vehicle-treated controls (Leung et al., 2018). This reduction in tumor size is due to the induction of apoptosis caused by deltarasin-mediated PDEδ inhibition. However, deltarasin treatment also leads to protective autophagy, indicating that blocking autophagy might enhance the efficacy of deltarasin (Leung et al., 2018). In support of this, the anti-autophagic drug 3-MA more effectively induces cancer cell death through apoptosis when combined with deltarasin in vitro than when the cells are treated with deltarasin alone (Leung et al., 2018). Similar results have also been reported when autophagy inhibitors are combined with ERK1/2 inhibitors (ERKi) in patient-derived pancreatic cancer xenograft models and when used in triple combination with the BRAF and CRAF (also known as RAF1) kinase inhibitors in KRAS-mutant cell lines (Bryant et al., 2019; Lee et al., 2019). For example, one study has shown that treating KRAS-mutant pancreatic cancer patient-derived xenograft models with ERKi alone decreased tumor weight twofold compared to that of vehicle-treated controls. By contrast, when ERKi were combined with the autophagy inhibitor hydroxychloroquine, they reduced tumor weight by approximately sixfold (Bryant et al., 2019). These results indicate that, although

Table 1. Stress-adaptation to oncogenic RAS-induced DNA damage and resulting therapeutic strategies

| DNA damage as a result of oncogenic RAS | Adaptation to DNA damage | Potential therapeutic strategies | References |
|---------------------------------------|--------------------------|---------------------------------|------------|
| Increased cell cycle entry and changes in DNA replication timing and activation | Sustained activity of the DNA damage response (DDR) protein ATR | Inhibiting the DDR pathway in combination with DNA-damaging agents | Murcia et al., 2019; Di Micco et al., 2006; Gilad et al., 2010; Schoppy et al., 2012 |
|                                       | Sustained activity of wild-type RAS isoforms in the cell | |
|                                       | Upregulation of the glucose importer GLUT1 | Inhibiting the DDR pathway in combination with metabolic inhibitors | Erber et al., 2019; Chen et al., 2001 |

Resulting cellular characteristics

| Increased levels of cytosolic DNA, aberrant replication fork progression, and increased genomic instability | Increased dependence on ATR, as evidenced by synthetic lethal knockout of ATR in RAS-transformed cells | Di Micco et al., 2006; Al Zubaidi et al., 2021; Gilad et al., 2010 |
| Activation of the DDR pathway, leading to cell death or oncogene-induced senescence | Increased sensitivity to DNA-damaging agents when wild-type HRAS and NRAS are knocked down in an oncogenic KRAS background | Di Micco et al., 2006; Bartkova et al., 2006; Grabocka et al., 2014 |
| | Increased dependence on metabolic processes, such as glucose import, as the combined inhibition of GLUT1 and either ATR or Chk1 (also known as CHEK1) results in increased apoptosis and a reduction in tumor volume in vivo | Erber et al., 2019 |
oncogenic RAS induces autophagy, inhibiting the RAS pathway can also lead to stress-adaptive autophagy. As such, pairing RAS pathway inhibitors with autophagy inhibitors might push a cancer cell towards programmed cell death instead of tumors-promoting autophagy responses.

In addition to the discovery that RAS pathway inhibition leads to protective autophagy, new insights into the cellular response to autophagy inhibitors have also come to light. A comprehensive pharmacological screen recently identified replication response inhibitors and the lysosome inhibitor chloroquine (CQ) as inducers of synthetic lethality in PDAC cells (Elliott et al., 2019). CQ has long been used to target lysosomal pathways, and inhibits the final stage of the autophagy response. This study revealed that reduced nucleotide biosynthesis in response to CQ treatment leads to replication stress, rendering the cells vulnerable to replication stress inhibitors. This phenotype was partially rescued by supplementation with aspartate, a precursor for de novo nucleotide synthesis (Elliott et al., 2019). These findings support the notion that commonly used drugs, such as autophagy inhibitors and membrane localization inhibitors of RAS, which have failed as monotherapies against RAS-driven cancers, may induce particular stress-adaptive responses that aid in a cancer’s survival and resistance to such therapies. Identifying these secondary stress responses may thus expose new targetable vulnerabilities while blocking such responses may revive old therapeutic strategies.

Macropinocytosis in drug response

Oncogenic RAS can be a potent inducer of macropinocytosis, depending on the type of oncogenic mutation involved (Fig. 2) (Hobbs et al., 2020). However, the KRASG12R mutant, which is rare in lung and colorectal cancer but more common in pancreatic cancer, is dispensable for the characteristic upregulation of macropinocytosis, as shown from the examination of ten different PDAC cell lines (Hobbs et al., 2020). This mutation causes a structural change in the protein that renders it incapable of binding to PI3Kα. A distinct PI3K isoform, p110γ (also known as PIK3CG), compensates for this loss and is responsible for the KRAS-independent upregulation of macropinocytosis in these cells (Hobbs et al., 2020). KRASG12R mutant cells are also more sensitive than KRASG12D/V cells to MEK/ERK and PI3Kγ inhibition (Hobbs et al., 2020). This increased sensitivity to PI3Kγ inhibition is most likely due to the inability of KRASG12R to activate the PI3K pathway, indicating that different RAS mutations might require specific therapeutic strategies to effectively target stress-adaptive pathways. Future work should include the investigation of compensatory mechanisms that result from structural and functional differences between RAS mutant subtypes, as this may help to lead to more individualized and effective treatments for RAS-driven cancers.

Although RAS mutants might employ different mechanisms to upregulate macropinocytosis, it is nevertheless elevated in most RAS-driven cancers. One idea, therefore, is to use this enhanced macropinocytosis as a system for delivering drugs, rather than trying to inhibit it (Liu and Ghosh, 2019). RAS mutant cancer cells preferentially scavenge lipids, glutamine and albumin through macropinocytosis (Liu and Ghosh, 2019). For example, cross-linked albumin nanoparticles are taken up in greater quantities by cells with oncogenic KRAS than by their wild-type counterparts, and colocalize with macropinosomes, indicating that macropinocytosis was the uptake mechanism (Liu and Ghosh, 2019). This system might therefore be used in the future to deliver drugs selectively to KRAS mutant cells, potentially reducing toxicity to non-transformed cells and enhancing treatment efficacy.

Macropinocytosis aids in cancer anabolism and can directly enhance resistance to anabolism-targeting therapies (Jayashankar and Edinger, 2020). Anabolism is the biosynthesis of macromolecules that support the metabolic needs of cells, and common therapies that target anabolism include gemcitabine, 5-fluorouracil (5-FU), doxorubicin and γ-irradiation (Jayashankar and Edinger, 2020). These drugs often kill cancer cells via necrosis, which is a sudden and pro-inflammatory form of cell death in which the contents of the dying cell are released into the surrounding environment. When surrounding cells undergo necrosis within the tumor microenvironment, RAS-mutant cancer cells use macropinocytosis to take up the macromolecular end products that form in the cellular debris to boost their nutrient supply. The presence of such debris can also reduce the sensitivity of macropinocytic oncogenic RAS cells to anabolism-targeting therapies, as seen in oncogenic pancreatic cancer cells, which lose their sensitivity to 5-FU when it is added alongside necrotic cellular debris. These cells showed proliferation levels similar to those of their untreated counterparts, whereas non-RAS mutant with low macropinocytosis cells remained sensitive to 5-FU. 5-[N-ethyl-N-isopropyl] amiroloride (EIPA) is a Na‘/H‘ exchanger inhibitor that blocks macropinocytosis without affecting receptor-mediated endocytosis. When cells were treated with 5-FU in the presence of necrotic cell debris and EIPA, the aforementioned survival advantage of RAS mutant cells was lost, indicating that necrotic cellular debris uptake had occurred via macropinocytosis (Jayashankar and Edinger, 2020). As the macropinocytosis-mediated uptake of macromolecules renders highly macropinocytic cancer cells tolerant to anabolic-targeting therapies, therapies that target both macropinocytosis and anabolic metabolism might provide a promising combination by which to block resistance mechanisms that emerge in the presence of anabolic-targeting therapies.

**ER stress adaptation in drug response**

In support of the importance of the UPR stress response in cancer cell stress tolerance and drug resistance, a recent drug-screening study identified inhibitors of heat shock protein 90 (HSP90)
proteins and AXL as the most detrimental to the growth of chemotherapy-naive parental control cell lines, compared to therapy-naive parental control cell lines (Yang et al., 2019). HSP90 proteins are chaperones responsible for proper protein folding, trafficking and degradation, and are involved in regulating the UPR response. AXL is a receptor tyrosine kinase that has been shown to activate the RAS pathway. Inhibitors of HSP90 and MEK, when combined, have strong anti-tumor effects in KRAS-mutant lung cancer patient-derived xenograft mouse models and in NSCLC xenograft mouse models, showing a three- to fourfold reduction in tumor weight compared to that of vehicle-treated controls (Yang et al., 2019). HSP90 inhibition has also been shown to preferentially induce apoptosis in KRAS-mutant colon cancer cells in vitro and in a colon cancer-derived xenograft model in nu/nu mice, indicating that this vulnerability might translate across different RAS-mutant tumor types (Wang et al., 2016). Targeting the UPR pathway can also block another stress-adaptive mechanism, protective autophagy, and can overcome resistance in melanoma cell lines, making the blocking of the UPR stress response an even more attractive approach (Ma et al., 2014). In the first example, protective autophagy was induced in response to BRAF inhibitors and blocked by the addition of a PERK inhibitor, leading to increased cell death. These findings suggest that blocking the UPR stress response might be an effective way to overcome this resistance mechanism (Ma et al., 2014). The same study also shows how stress-adaptive pathways are often interlinked, and how identifying and targeting the most critical mechanism for a cell could reduce its overall stress tolerance. In addition, it might be possible to identify which patients would most benefit by UPR-based combinatory therapies by assessing their levels of UPR activity (Yang et al., 2019). Thus, stress-adaptive pathways could be used as biomarkers to predict patient responses to specific stress-targeting therapies and to predict which resistance mechanisms might emerge by profiling the stress-adaptive responses that are already heightened at the start of treatment.

Adaptation to pan-stress stimuli in drug response

As previously described, oncogenic KRAS signals upregulate SG formation via the production of the signaling molecule 15-d-PGJ2. This process promotes survival in response to a variety of RAS- and chemotherapy-induced stresses. For example, in oncogenic KRAS-expressing HeLa cells, levels of oxidative stress-induced apoptosis increased following the addition of the SG inhibitor emetine (Grabocka and Bar-Sagi, 2016). By contrast, apoptosis levels in wild-type HeLa cells remained unaffected by emetine treatment, indicating that SGs play a specific role in survival during stress in oncogenic KRAS-driven cells (Grabocka and Bar-Sagi, 2016). When SG formation was blocked using a COX-1/2 inhibitor in oncogenic KRAS-driven colon cancer cells, the cells also showed increased sensitivity to the chemotherapeutic drug oxaliplatin. This effect functioned at a paracrine level, consistent with the paracrine induction of SGs by oncogenic KRAS (Grabocka and Bar-Sagi, 2016). Multiple anti-cancer drugs have been shown to induce SGs, including 5-FU, lapatinib, sorafenib, oxaliplatin, bortezomib and selenite, to name a few (Kaehler et al., 2014; Adjibade et al., 2020; Hu et al., 2021). One study reported that 5-FU treatment of colorectal cancer cell lines in vitro increased their expression of Musashi-1, a colon stem cell marker and RNA-binding protein, which contributed to the formation of anti-apoptotic SGs and to the population of CD44+ stem cells (Chiou et al., 2017). In ovarian carcinoma cells, the inhibition of Musashi-1 blocked paclitaxel resistance, implicating this SG-promoting protein in drug resistance (Chen et al., 2019). Given that SGs are a mechanism of resistance induced by both oncogenic KRAS and chemotherapy, with the former also creating resistance in a paracrine manner in surrounding tissue, SG-targeting agents are likely to provide potent therapeutics for treating oncogenic RAS-driven tumors.

Other proteins or pathways might also respond to a multitude of RAS-induced stresses. One such example has been identified in the investigation of resistance mechanisms that accompany treatment with EGFR inhibitors. Oncogenic RAS colorectal cancer cells that are sensitive to EGFR-targeting antibodies undergo apoptosis through the p73-dependent transcriptional activation of the BH3-only protein PUMA (also known as BBC3); when these cells acquire resistance, they exhibit a reduction in PUMA expression (Knickelbein et al., 2018). PUMA induces apoptosis in response to ER and genotoxic stress, and to deregulated oncogenic signaling (Yu and Zhang, 2008). Thus, PUMA loss might be a stress-adaptive mechanism that promotes survival in the context of many oncogenic RAS-induced stresses. The reactivation of PUMA, when combined with RAS pathway inhibition, might produce a synergistic effect that promotes apoptosis and reduces the survival of RAS-driven tumor cells. Another potential strategy would be to induce PUMA alongside inhibiting autophagy, because autophagy protects against many oncogenic RAS-induced stresses. Overall, it is apparent that some oncogenic RAS-induced, and therapy-derived, stress-adaptive mechanisms lead to a stress-tolerant state that mitigates against a plethora of RAS-induced stresses. As such, the identification and targeting of such mechanisms might be the most effective way to enhance the efficacy of RAS-targeted therapies.

Emerging resistance to KRASG12C inhibitors

The mechanisms that underlie resistance to KRASG12C inhibitors in lung and other types of cancer are at an early stage of investigation. Thus far, a variety of resistance mechanisms have been described, but most seem to share the common end result of reactivating the MAPK pathway (Ryan et al., 2020; Xue et al., 2020; Tanaka et al., 2021), such as acquired mutations in BRAF, NRAS, MAP2K1 (MEK1) and KRAS itself (Tanaka et al., 2021). These acquired KRAS mutations include other common variants in KRAS that are seen across mutant KRAS-driven cancers, such as the G13D and G12V substitutions, as well as a novel mutation in residue 96 (KRASY96D) that are yet to be documented in the clinic (Tanaka et al., 2021). Unfortunately, many of these acquired resistance mechanisms were identified from the biopsies of a single patient, indicating that resistance to KRASG12C inhibitors is quite heterogeneous. This would suggest that there may be an even greater level of heterogeneity within the patient population. Therefore, there exists a great need for a more generalized approach to blocking the reactivation of MAPK signaling during treatment with KRASG12C inhibitors. Because many of the stress-adaptive mechanisms described above are activated through MAPK signaling, it is likely that they also play a role in resistance to KRASG12C inhibitors. Thus, investigating the role of stress-adaptive mechanisms in this process may provide insight into strategies to prevent and overcome emerging resistance to KRASG12C inhibitors.

Overall, as new therapies arise that aim to target oncogenic RAS, the integrated stress response of the cell should be considered in terms of investigating resistance mechanisms, combining therapies and identifying biomarkers, in order to block resistance and enhance patient outcomes.

Conclusions

The findings we discuss here indicate that although tumor resistance is multifactorial, stress-adaptive mechanisms might provide key
targetable vulnerabilities in RAS-driven tumors. From a therapeutic perspective, the combinatorial inhibition of RAS, its downstream signaling pathways, multiple stress-response pathways and/or adaptive mechanisms to pan-stress stimuli, provide a promising approach to the treatment of these tumors. Perhaps, these combinations could be stratified based on which stress-response pathways are known to be activated among different RAS-driven cancers or as a result of RAS-targeted therapies. The upregulation of stress-response pathways might also be used as biomarkers of resistance, as well as of responses to specific therapies. Therapies that have previously failed in the clinic might also regain clinical traction, particularly once the stress-adaptive pathways or proteins that aid in a specific resistance mechanisms to a therapy are identified. It is exciting to consider the possibility that RAS-driven stress-adaptive mechanisms could provide a promising new avenue of investigation for therapeutics that alone or in combinations could successfully treat RAS-driven cancers.

This article is part of a collection ‘The RAS Pathway: Diseases, Therapeutics and Beyond’, which was launched in a dedicated Special Issue guest edited by Donita Brady and Arvin Dar. See related articles in this collection at https://journals.biologists.com/dmm/collection/5089/The-RAS-Pathway.

Acknowledgements
We thank all members of the Grabocka laboratory for helpful discussions.

Competing interests
The authors declare no competing or financial interests.

Funding
This work was supported by a National Cancer Center Support Grant (P30 CA056036) to the Sidney Kimmel Cancer Center at Thomas Jefferson University; National Cancer Institute R37 CA320645, American Cancer Society 103042-IRG-16-2444-10-IRG and Margaret Q. Landenberger Research Foundation grants to E.G.; and National Cancer Institute RO1 CA610495 to A.E.A.

References
Acosta-Alvear, D., Zhou, Y., Blais, A., Tsikitis, M., Lents, N. H., Arias, C., Podolsky, M. A. and Glick, A. B. (2019). Oncogene-induced senescence is a DNA damage response triggered by DNA replication stress phenotype of KRAS mutant cancer cells. Sci. Rep. 9, 11651. doi:10.1038/s41598-019-44440-y
Alves, S., Castro, L., Fernandes, M. S., Francisco, R., Castro, P., Priauit, M., Chaves, S. R., Moyer, M. P., Oliveira, C., Seruca, R. et al. (2015). Colorectal cancer-related mutant KRAS alleles function as positive regulators of autophagy. Oncotarget 6, 30787-30802. doi:10.18632/oncotarget.5021
Adjibade, P., Simoneau, B., Ledoux, N., Gauthier, W.-N., Nkurunzziza, M., Khajurian, E. W. and Mzroui, R. (2020). Treatment of cancer cells with Lapatinib negatively regulates general translation and induces stress granules formation. PLoS ONE 15, e0231894. doi:10.1371/journal.pone.0231894
Al Zubaidi, T., Gehrisch, O. H. F., Genois, M.-M., Liu, Q., Lu, S., Kung, J., Xie, Y., Chiou, G.-Y., Yang, T.-W., Huang, C.-C., Tang, C.-Y., Yen, J.-Y., Tsai, M.-C., Chen, H.-Y., Fadhilah, N., Lin, C.-C. and Jong, Y.-J. (2017). Musashi-1 promotes a cancer stem cell lineage and chemoresistance in colorectal cancer cells. Sci. Rep. 7, 2172. doi:10.1038/s41598-017-02057-9
Alzheimer, K., Fukuoka, H., Imagoh-Omhi, S., Saito, H. and Takekawa, M. (2008). Formation of stress granules inhibits apoptosis by suppressing stress-responsive regulatory protein 78 (GRP78) regulates redox status in pancreatic cancer thereby maintaining “stemness”. Cell Death Dis. 10, 132. doi:10.1038/s41419-019-1408-5
Arendt, J. C., Zelinka, M., Zeilhofer, H. U., Podolsky, M. A. and Glick, A. B. (2017). ER stress and distinct outputs of the IRE1α RNSase control proliferation and senescence in response to oncogenic Ras. Proc. Natl Acad. Sci. USA 114, 9900-9905. doi:10.1073/pnas.1701757114
Fan, S., Meng, Q., Laterra, J. J. and Rosen, E. M. (2007). Ras effector pathways modulate scatter factor-stimulated NF-κB signaling and protection against DNA damage. Oncogene 26, 4774-4796. doi:10.1038/onc.2007.262171.

Fan, S., Meng, Q., Laterra, J. J. and Rosen, E. M. (2009). Role of Src signal transduction pathways in scatter factor-mediated cellular protection. J. Biol. Chem. 284, 7561-7577. doi:10.1074/jbc.M007497200.

Fernández-Sánchez, M. E., Barbier, S., Whitehead, J., Béalle, G., Michel, A., Latorre-Ossa, H., Rey, C., Fouassier, L., Claperson, A., Brullé, L. et al. (2015). Mechanical induction of the tumorigenic β-catenin pathway by tumour growth. Nature 518, 523-526. doi:10.1038/nature14055.

Forsythe, J. A., Jiang, B. H., Iyer, N. V., Agani, F., Leung, S. W., Koos, R. D. and Guo, J. Y., Chen, H.-Y., Mathew, R., Fan, J., Strohecker, A. M., Karsli-Grabocka, E., Pylayeva-Gupta, Y., Mathew, K. J. M., Lubkov, V., et al. (2018). Alterations of mTOR signaling impact metabolic stress resistance in colorectal carcinomas with BRAF and KRAS mutations. Sci. Rep. 8, 9204. doi:10.1038/s41598-018-27394-1.

Gilad, O., Nabet, B. Y., Ragland, R. L., Schoopy, D. W., Smith, K. D., Dunchak, C. A., Chen, G., Price, S., Lu, W., Teng, X. et al. (2010). Combining ATR suppression with oncogenic Ras Synergistically increases genomic instability, causing synthetic lethality or tumorigenesis in a dosage-dependent manner. Cancer Res. 70, 9693-9702. doi:10.1158/0008-5472.CAN-10-2286.

Hobbs, G. A., Baker, N. M., Miermont, A. M., Thurman, R. D., Pierobon, M., Hill, R., Li, Y., Tran, L. M., Dry, S., Calvopina, J. H., Garcia, A., Kim, C., Wang, Y., et al. (2015). Molecular pathways: hypoxia and suppress the HIF-1 pathway. Cancer Discovery 5, 167-180. doi:10.1158/2159-8290.CD-15-0559.

Jääne, P. A., Rybkin, I. I., Spira, A. I., Riey, G. J., Papadopoulos, K. P., Sabari, J. K., Johnson, M. L., Heist, R. S., Bазенов, L., Barve, M. et al. (2020). KRYS1L-1: activity and safety of adagrasib (MRTX849) in advanced/metastatic non-sm–small cell lung cancer (NSCLC) harboring KRAS G12C mutation. Eur. J. Cancer 130, 51-52. doi:10.1016/j.ejca.2020.03.076.

Jayashankar, Y. and Edinger, A. L. (2020). Macropinosysis confers resistance to therapies targeting cancer anabolism. Nat. Commun. 11, 1211. doi:10.1038/s41467-020-14923-8.

Kadowaki, H. and Nishitoh, H. (2013). Signaling pathways from the endoplasmic reticulum and their roles in disease. Genes 4, 306-333. doi:10.3939/genes040306.

Kaehler, C., Isensee, J., Hucho, T., Lehrach, H. and Krobitsch, S. (2014). 5'-Flurouracil affects assembly of stress granules based on RNA incorporation. Nucleic Acids Res. 42, 6436-6447. doi:10.1093/nar/gku264.

Kamphorst, J. J., Cross, J. R., Fan, J., De Stanchina, E., Mathew, R., White, E. P., Thompson, C. B. and Campisi, J. (2013). Hypoxic and Ras-transformed cells support growth by scavenging unsaturated fatty acids from lysophospholipids. Proc. Natl Acad. Sci. USA 110, 8882-8887. doi:10.1073/pnas.1307237110.

Kamphorst, J. J., Nofal, M., Commizzo, C., Hackett, S. R., Lu, W., Grabocka, E., Vahedi, H., Miller, G., Derbir, J. A., Bar–Sagi, D. et al. (2015). Human pancreatic cancer tumours are nutrient poor and tumour cells actively scavenge extracellular protein. Cancer Res. 75, 544-553. doi:10.1158/0008-5472.CAN-14-2211.

Kang, R., Hou, W., Zhang, Q., Chen, R., Lee, J. Y., Bartlett, D. L., Lotze, M. T., Tang, D. and Zeh, H. J. (2014). RAGE is essential for oncogenic KRAS-mediated hypoxic signaling in pancreatic cancer. Cell Death Dis. 5, e1480-e1480. doi:10.1038/cddis.2014.445.

Kikuchi, H., Pino, M. S., Zeng, M., Shirasawa, S. and Chung, D. C. (2009). Oncogenic KRAS and BRAF differentially regulate hypoxia-inducible factor-1α and -2α in colon cancer. Cancer Res. 69, 8499-8506. doi:10.1158/0008-5472.CAN-09-2213.

Kinsey, C. G., Camolotto, S. A., Boespflug, A. M., Guillen, K. P., Foth, T., Truong, A., Schuman, S. S., Shea, J. E., Seipp, M. T., Yap, J. T. et al. (2019). Protective autophagy elicited by RAF→MEK→ERK inhibition suggests a treatment strategy for RAS-driven cancers. Nat. Med. 25, 620-627. doi:10.1038/s41591-019-0367-9.

Knickelbein, K., Tong, J., Chen, D., Wang, Y.-J., Misale, S., Bardelli, A., Yu, J. and Zhang, L. (2018). Restoring PUMA induction overcomes KRAS-mediated resistance to anti-EGFR antibodies in colorectal cancer. Oncogene 37, 4551-4561. doi:10.1038/s41388-018-0289-x.

Krushna, C., Wang, Q., Bhaskar, P. T., Miller, L., Wang, Z., Wheaton, W., Chandel, N., Laakso, M., Muller, W. J., Allen, L. E. et al. (2013). Hexokinase 2 is required for tumor initiation and maintenance and its systemic deletion is therapeutic in mouse models of cancer. Cancer Cell 24, 213-228. doi:10.1016/j.ccr.2013.06.014.

Kurppa, K. J., Liu, Y., To, C., Zhang, T., Fan, M., Vajdi, A., Knelson, E. H., Xie, Y., Lim, K., Cejas, P., et al. (2020). Treatment-induced tumor dormancy through YAP-mediated transcriptional reprogramming of the apoptotic pathway. Cancer Cell 37, 104-122.e12. doi:10.1016/j.ccell.2019.12.006.

Lee, C.-J., Lee, M.-H., Lee, J.-Y., Song, J. H., Lee, H. S. and Cho, Y.-Y. (2013). RSK2-induced stress tolerance enhances cell survival signals mediated by inhibition of GSK3α activity. Biochem. Biophys. Res. Commun. 440, 112-118. doi:10.1016/j.bbrc.2013.09.042.

Lee, C.-S., Lee, L. C., Yuan, T. L., Chakka, S., Fellmann, C., Lowe, S. W., Caplen, N. J., Mccormick, F. and Luo, J. (2019). MAP kinase and autophagy pathways cooperate to maintain RAS mutant cancer cell survival. Proc. Natl Acad. Sci. USA 116, 4508-4517. doi:10.1073/pnas.1803419116.

Leung, E. L. H., Luo, L. X., Liu, Z. Q., Wong, V. K., Wu, L. L., Xie, Y., Zhang, N., Qu, Q. Y., Fan, X., Li, Y., et al. (2018). Inhibition of KRAS-dependent lung cancer cell growth by deltarasin: blockage of autophagy increases its cytotoxicity. Cell Death Dis. 9, 121. doi:10.1038/s41419-017-0080-z.

Li, X.-Z., Zhang, H.-S., Xu, Y.-M., Zhang, R.-J., Chen, Y., Fan, L., Qin, Y.-Q., Liu, Y., Li, M. and Fang, J. (2017). Knockdown of IRE1α inhibits colon tumorigenesis through decreasing β-catenin and IRE1α targeting suppresses colon cancer cells. Oncogene 36, 6738-6746. doi:10.1038/onc.2017.284.

Li, X., Zhang, J., Zhang, S., Li, J. and Du, W. (2020). Overexpression of G3BP1 facilitates the progression of colon cancer by activating β-catenin signaling. Mol. Med. Rep. 22, 4403-4411. doi:10.3892/mmr.2020.11527.
Li, B., Skoulidis, F., Falchuk, G., Sacher, A., Velcheti, V., Dy, G., Price, T., Borghaei, H., Schuler, M., Kato, T. et al. (2021a). PSA1.07. Registration phase 2 trial of sotorasib in KRAS p.G12C mutant NSCLC: first disclosure of the Codebreak 100 primary analysis. J. Thorac. Oncol. 16, 561. doi:10.1016/j.jto.2021.02.015

Li, L. Y., Guan, Y. D., Chen, X. S., Yang, J. M. and Cheng, Y. (2021b). DNA repair pathways in cancer therapy and resistance. Front. Pharmacol. 11, 629266. doi:10.3389/fphar.2021.629266

Liberti, M. V. and Locasale, J. W. (2016). The Warburg effect: how does it benefit cancer cells? Trends Biochem. Sci. 41, 211-218. doi:10.1016/j.tibs.2015.12.001

Lin, C.-G., Jang, J. and Kim, C. (2018). Cellular machinery for sensing mechanical force. BMB Reports 51, 623-629. doi:10.5483/BMBRep.2018.51.12.237

Lin, H.-H., Lin, H.-K., Lin, I.-H., Chiu, Y.-W., Chen, H.-W., Liu, C.-Y., Harn, H.-L., Chiu, W.-T., Wang, Y.-K., Shen, M.-R. et al. (2015). Mechanical phenotype of cancer cells: cell softening and loss of stiffness sensing. Oncotarget 6, 98320-98324. doi:10.18632/oncotarget.4173

Li, G.-Y., Döpppel, H., DelGiorno, K. E., Zhang, L., Leitges, M., Crawford, H. C., Murphy, M. P. and Storz, P. (2016). Mutant KRAs-induced mitochondrial oxidative stress in acinar cells upregulates EGFR signaling to drive formation of pancreatic precancerous lesions. Cell Reports 14, 2325-2336. doi:10.1016/j.celrep.2016.02.029

Liu, X. and Ghosh, D. (2019). Intracellular nanoparticle delivery by oncogenic KRAS-mediated macropinocytosis. Int. J. Nanomedicine 14, 6589-6600. doi:10.2147/IJN.S212861

Liu, Y.-J., Fan, X.-Y., Wang, A.-D., Xia, Y.-Z., Fu, W.-R., Liu, J.-Y., Jiang, F.-L. and Luo, J., Emanuele, M. J., Li, D., Creighton, C. J., Schlabach, M. R., Li, L. Y., Guan, Y. D., Chen, X. S., Yang, J. M. and Cheng, Y. (2019). CB-839, a glutaminase inhibitor, in combination with cabozantinib in renal cell carcinoma. Cancer Res. 80, 1630-1643. doi:10.1158/0008-5472.CAN-19-1363

Mirta, R. L., Risse, J., Swinnen, J. V. and Zandi, N. (2019). Lipid metabolism in cancer cells under metabolic stress. Br. J. Cancer 120, 1090-1098. doi:10.1038/s41416-019-0451-4

Murcia, L., Clemente-Ruiz, M., Pierre-Elies, P., Royou, A. and Milan, M. (2019). Selective killing of RAS-malignant tissues by exploiting oncogenic-induced DNA damage response activation. Cell Death. Differ. 26, 998-1012. doi:10.1038/cdd.2016.14

Ober, S. G., Voss, M. H., Reinhard, C. H., Lipper, C. H., Lee, W., Johnson, C., Sholl, L. M., South, A. P., Marto, J. A. et al. (2020). IERS, a DNA damage response gene, is required for Notch-mediated induction of squamous cell differentiation. elife 9, e58081. doi:10.7554/eLife.58081

Park, M. T., Kim, M. J., Suh, Y., Kim, R.-K., Kim, H., Lim, E.-J., Yoo, K.-C., Lee, G.-H., Kim, Y.-H., Hwang, S.-G. et al. (2014). Novel signaling axis for ROS generation during K-Ras-induced cellular senescence. Cell Death. Differ. 21, 1185-1197. doi:10.1038/cdd.2014.34

Park-Perez, L., Despouy, G., Delage-Mourroux, R. and Boyer-Guitart, M. (2015). Interplay between ROS and autophagy in cancer cells, from tumor initiation to cancer therapy. Redox Biol. 4, 184-192. doi:10.1016/j.redox.2014.12.003

Prior, I. A., Hood, F. E. and Hartley, J. L. (2020). The frequency of Ras mutations in cancer. Cancer Res. 80, 2967-2974. doi:10.1158/0008-5472.CAN-19-3682

Qian, Y., Ye, S., Chen, Y., Epstein, J., Davies, F. E., Morgan, G., Walker, B. A. and Rhee, F. (2019). Mutant KRAS enhances stress granules and resistance to proteasome inhibition via d-pPGJ2 in multiple myeloma. Blood 143, 4383-4383. doi:10.1182/blood-2019-130493

Razzaghli, H., Troester, M. A., Gierach, G. L., Olshan, A. F., Yankaskas, B. C. and Tanguay, R. M., Mazroui, R. and Khandjian, E. W. (2019). RAD51 is a potential marker for prognosis and regulates DNA repair in breast cancer cells. Cancer Discov. 9, 1536-1551. doi:10.1158/2155-9555_CD-18-0456

Reitman, Z. J., Jin, G., Karoly, E. D., Spasojevic, I., Yang, J., Kinzler, K. W., He, Y., Biggin, D. D., Vogelstein, B. and Yan, H. (2011). Profiling the effects of isocitrate dehydrogenase 1 and 2 mutations on the cellular metabolome. Proc. Natl Acad. Sci. USA 108, 3270-3275. doi:10.1073/pnas.1007393108

Ren, Z. G. (2018). RAD51 is a potential marker for prognosis and regulates proliferation in pancreatic cancer. Int. J. Radiat. Oncol. Biol. Phys. 102, e159. doi:10.1016/j.ijrobp.2018.07.613

Ryan, M. B., Fece De La Cruz, F., Phat, S., Myers, D. T., Wong, E., Shahzade, H. A., Hong, C. B. and Corcoran, R. B. (2020). Vertical pathway inhibition overcomes adaptive feedback resistance to KRASG12C inhibition. Clin. Cancer Res. 26, 1633-1643. doi:10.1158/1078-0432.CCR-19-3523

Schlabach, M. R., Million, S. Z., Li, A. R., Grassian, A. R., Liao, Z., Gerhard-Hines, Z., Irie, H. Y., Gao, S., Puigserver, and Brugge, J. S. (2009). Antioxidant and oncogene rescue of metabolic defects caused by loss of macropinocytosis. Nature 461, 109-113. doi:10.1038/nature08268

Scoppito, D. W., Ragland, R. L., Gilad, O., Shastri, N., Peters, A. A., Murga, M., Fadini, P. J., Rapp, K. J., Machtens, J., Apollonio, O., Dapino, M. E. and D’Agostino, E. (2015). Oncogenic stress sensitizes murine cancers to hypomorphogenic suppression of AT R. J. Clin. Investig. 122, 241-252. doi:10.1172/JCI85928
Sen, T., Tong, P., Diao, L., Li, L., Fan, Y., Hoff, J., Heymach, J. V., Wang, J. and Byers, L. A. (2017). Targeting AXL and mTOR pathway overcomes primary and acquired resistance to WEE1 inhibition in small-cell lung cancer. Clin. Cancer Res. 23, 6239-6253. doi: 10.1158/1078-0432.CCR-17-1284

Sharma, S., Lee, D., Li, B., Quinlan, D., Takahashi, F., Maheswaran, S., McDermott, U., Azizian, N., Zou, L., Fischbach, M. et al. (2010). A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 141, 69-80. doi: 10.1016/j.cell.2010.02.027

Shi, Z., Yu, X., Yuan, M., Lv, W., Feng, T., Bai, R. and Zhong, H. (2019). Activation of the PERK-AIF4 pathway promotes chemo-resistance in colon cancer cells. Sci. Rep. 9, 3210. doi: 10.1038/s41598-019-39547-x

Shirazi, F., Jones, R. J., Singh, R. K., Zou, J., Kuiatse, I., Berkova, Z., Wang, H., Lee, H. C., Hong, S., Dick, L. et al. (2020). Activating KRAS, NRAS, and BRAF mutants enhance proteasome capacity and reduce endoplasmic reticulum stress in multiple myeloma. Proc. Natl Acad. Sci. USA 117, 20004-20014. doi: 10.1073/pnas.200502117

Sim, E., Irollo, E. and Grabocka, E. (2019). Evaluating stress granules in pancreatic cancer in vitro and in vivo: in: methods in molecular biology. Methods Mol. Biol. 1882, 183-195. doi: 10.1007/978-1-4939-8979-2_17

Smith, M. J., Neel, B. G. and Ikura, M. (2013). NMR-based functional profiling of RASopathies and oncogenic RAS mutations. Proc. Natl Acad. Sci. USA 110, 4547-4579. doi: 10.1073/pnas.1218173110

Somasekharan, S. P., El-Naggar, A., Leprivier, G., Cheng, H., Hajee, S., Smith, M. J., Neel, B. G. and Ikura, M. (2015). YB-1 regulates stress granule formation and tumor progression by translationally activating G3BP1. J. Cell Biol. 208, 913-929. doi: 10.1083/jcb.201411047

Son, J., Lysiotis, C. A., Ying, H., Wang, X., Hua, S., Ligorio, M., Perera, R. M., Ferrone, C. R., Mullarky, E., Shyh-Chang, N. et al. (2015). Translationally activating G3BP1. Mol. Cell. 60, 26-47. doi: 10.1016/j.molcel.2014.09.046

Sun, Z., Schwenzer, A., Rupp, T., Mardamoothoo, D., Vegliante, R., Lefebvre, O., Klein, A., Hussenet, T. and Orend, G. (2016). Tenasin-C promotes human melanoma metastasis through integrin α6β1-mediated YAP inhibition. Cancer Res. 76, 950-961. doi: 10.1158/0008-5472.CAN-17-1597

Sun, Z., Velázquez-Quesada, I., Mardamoothoo, D., Ahowesso, C., Yilmaz, A., Spenlé, C., Averous, G., Erne, W., Oberndorfer, F., Oszwald, A. et al. (2019). Tenasin-C promotes human melanoma metastasis through integrin α6β1-mediated YAP inhibition. Cancer Res. 79, 950-961. doi: 10.1158/0008-5472.CAN-19-1120

Yu, J. L., Rak, J. W., Klement, G. and Kerbel, R. S. (2014). Force-dependent transformation of human surface ovarian epithelial cells revealed by functional proteomics and mass spectrometry. Cancer Res. 74, 6239-6253. doi: 10.1158/0008-5472.CAN-13-3578

Zou, J., Kuiatse, I., Berkova, Z., Wang, H., Lee, H. C., Hong, S. and Dick, L. (2019). Activating KRAS, NRAS, and BRAF mutants enhance proteasome capacity and reduce endoplasmic reticulum stress in multiple myeloma. Cell 173, 26-47. doi: 10.1016/j.cell.2018.07.031