

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Molecular Mechanisms Involved in the Acquisition of Resistance to Treatment of Colon Cancer Cells

Dora M. Velázquez,
María Cristina Castañeda-Patlán and
Martha Robles-Flores

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/63524>

Abstract

Cancer cells are remarkably resilient to therapies aimed at their elimination. The exploration of pathways that sustain cancer cells and that allow cancer cells to become resistant has revealed new avenues for chemotherapeutic development, as well as rational approaches to combination therapies based on existing treatment options. Several signaling pathways, such as Wnt, phosphoinositide 3-kinase (PI3K), and Ras-Raf-MEK, constitute integrated networks that work together to maintain cellular homeostasis under basal conditions and to drive cell-mass accumulation and cell cycle progression in the presence of appropriate mitogenic stimuli. During cancer development, these pathways are corrupted in malignant cells to maintain viability and proliferative activity under environmentally stressful conditions such as limited growth factors, oxygen, and nutrients that drive normal cells into quiescence or death. Importantly, dysfunction within any one of these pathways results in compensatory responses from the other networks. Thus, biological research is gradually shifting toward more general approaches that target entire pathways rather than isolated components and integrate those pathways into biological networks.

Keywords: cancer resistance, autophagy, hypoxia, survival signaling pathways, colon cancer

1. Introduction

The inherent or developed resistance of many cancer cells to chemotherapy, targeted types of therapy, and irradiation is the primary cause for treatment failure in clinical oncology. Through basic research efforts aimed at gaining a better understanding of the mechanisms responsible for these effects, it is hoped that more effective strategies to manage this disease will be developed.

Wnt signaling has been recognized as one of the most important contributors to malignant transformation in many types of solid tumors. Canonical Wnt signaling is altered in most cases of colorectal cancer (CRC). Indeed, a great amount of experimental evidence has shown that mutations in the adenomatous polyposis coli (APC) gene, a key negative modulator of canonical Wnt signaling, trigger the molecular pathogenesis of this type of cancer [1, 2]. The Wnt pathway has also been demonstrated to play an important role in the regulation of adult stem cell systems, and canonical Wnt signaling regulates the self-renewal and maintenance of both stem and cancer stem cells (CSCs). Cross talk has been reported between canonical Wnt signaling and hypoxia-inducible factor (HIF) signaling in tumor progression and metastasis [3]. However, the molecular mechanisms involved in this cross talk remain poorly understood.

Diminished oxygen availability (hypoxia) with the hypoxia-inducible factors (HIFs) mediating adaptation to it causes autophagy establishment, particularly in RAS-driven and BRAF-driven cancers, such as in most colorectal carcinomas. However, the relevance of the relation between HIFs and autophagy in drug resistance is not well understood. We have examined the effects of stable knockdown of HIF-1 α or HIF-2 α expression on malignant phenotype maintenance and in the canonical Wnt activation (β -catenin dependent). Our results indicated that although both HIF-1 α and HIF-2 α are essential for stemness and malignancy maintenance, these two proteins exert different effects and play opposing roles in canonical Wnt signaling [3]. We have also examined the effects of HIFs silencing on autophagy and drug resistance displayed by cancer cells. In agreement with other groups, we have observed that cancer cells exhibit high basal levels of autophagy and co-expression of HIF-1 α and HIF-2 α , compared with control nonmalignant cells and that the combination of mTOR inhibition with the autophagy inhibitor hydroxychloroquine (HCQ) dramatically induced cell death via apoptosis [4].

In addition, it has been demonstrated recently that challenging cancer cells with stresses that they would typically encounter during tumor progression or as a part of a therapeutic regimen to treat or manage the disease, including chemotherapeutic agents, gamma irradiation, and hypoxic and serum-limiting conditions, increase the rate of microvesicles (MVs, referred also as oncosomes) formation and shedding by cells [5, 6]. These MVs are secreted from numerous types of cells and function in intercellular communication by transporting intracellular contents, such as protein, mRNA, and microRNAs (miRNAs) [7]. Oncosomes secreted by cancer cells may play an important role in cancer progression by promoting angiogenesis, neutrophil infiltration, and the education of bone marrow-derived cells [8]. Indeed, recent findings suggest that MVs can promote cell survival and contribute to drug resistance.

In this review we focus on the molecular mechanisms whereby cancer cells develop resistance to antineoplastic drugs, particularly focused in the cell signaling pathways involved. A better

understanding of the mechanisms that drive such resistance would enable the development of approaches to overcome it.

2. Survival signaling pathways: highly integrated cellular networks

Targeted blockade of aberrantly activated signaling pathways is an attractive therapeutic strategy for solid tumors, but drug resistance is common. Several selective inhibitors, particularly against kinases and receptor tyrosine kinases (RTKs), have shown promising initial efficacy [9]; however, with few exceptions, the duration of response is limited; drug resistance rapidly emerges. This underscores the difficulty of successfully treating an adept, heterogeneous disease with a single targeted therapy and highlights the fact that monotherapy is often not a tractable long-term therapeutic approach.

Resistance mechanisms can include alterations in the drug target itself, the pathway in which the target signals, or a parallel pathway that can alleviate the pressure on the cell due to blockade of the target [10]. Alternatively, drug resistance may be mediated by epigenetic reprogramming [11], by epithelial-to-mesenchymal transition (EMT) [12], or by emergence of a less differentiated, progenitor cell type [13]. Inducers of EMT, such as growth factors, transforming growth factor beta (TGF β), and Wnt ligands, induces the expression of a gene program that leads to the suppression of the expression of the cell adhesion protein E-cadherin via the expression of transcriptional repressors such as Snail, Slug, Zeb1, Zeb2, and Twist. Besides these genes, other typical markers of EMT are N-cadherin, vimentin, and fibronectin-1, which are usually expressed in mesenchymal cells [3].

The emergence of acquired resistance to targeted therapy against cancer is very common and the most frequent cause of treatment failure in cancer patients. This resistance can be mediated by signaling pathway reactivation [14] or by genetic or epigenetic events occurring within cancer cells [10]. Ultimately, these events lead to the activation of growth and survival signaling pathways within cancer cells that enable them to survive the stressful conditions. However, for most drugs, the identities of potential resistance pathways are unknown. The signaling pathways more frequently associated with cancer resistance to treatment are the following:

2.1. The PI3K signaling network

The phosphoinositide 3-kinase (PI3K) signaling pathway, which lies downstream of various growth factor receptor tyrosine kinases, including the EGFR, is often aberrantly activated in human cancers. It plays critical roles in the regulation of cell growth, proliferation, differentiation, motility, survival, and intracellular trafficking. When deregulated, it is a major driver of tissue hyperplasia, oncogenesis [15], and is implicated in many aspects of tumorigenesis, including inappropriate cellular proliferation, angiogenesis, metastasis, and resistance to cell death.

There are three classes of PI3K enzymes: the Class I PI3Ks play a central role in the transmission of regulatory signals through the metabolism-signaling supernetwork [15]. Class I PI3Ks are

expressed as heterodimers consisting of four p110 catalytic subunits and one of two p85 regulatory subunits and are activated primarily by tyrosine kinase signaling pathways and heterotrimeric G protein-coupled signaling pathways. Upon activation, Class I PI3Ks preferentially phosphorylate PI-4,5-P2 to yield the second messenger PI-3,4,5-P3 [15]. The activation of PI3K is dampened by the lipid phosphatase PTEN, which dephosphorylates PI-3,4,5-P3 to regenerate PI-4,5-P2, thereby disengaging the proximal signaling proteins from the network. The local accumulation of PI-3,4,5-P3 at the inner leaflet of the plasma membrane attracts proteins containing pleckstrin homology (PH) domains, which bind PI-3,4,5-P3 and PI-3,4-P2 to act as proximal signal transducers in the PI3K pathway [15] (**Figure 1**).

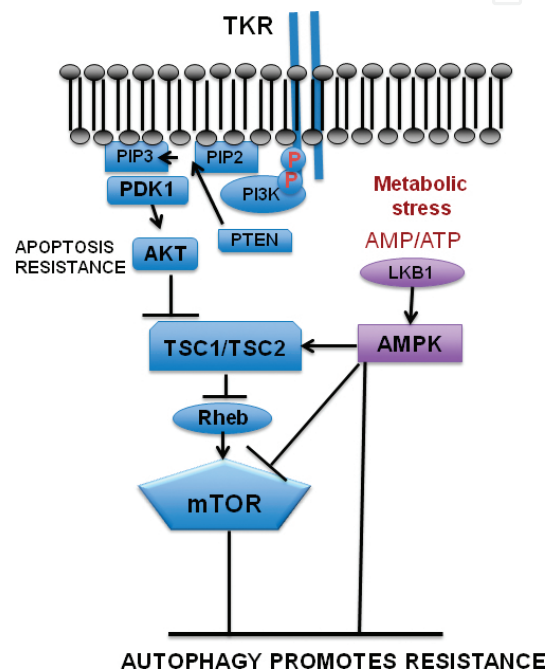


Figure 1. The PI3K and AMPK pathways are shown. Different members of the PI3K pathway increase apoptosis resistance. AMPK also regulates cellular metabolism by phosphorylating directly the tumor suppressor tuberous sclerosis 1 and 2 complex (TSC1/TSC2) to activate mTOR via Rheb GTPase.

The best-studied member of this set of PH domain-containing proteins is the serine-threonine kinase AKT, which is juxtaposed with its upstream activating protein kinase, PDK1. AKT is activated through phosphorylation of its threonine 308 residue by PDK1 and of serine 473 by mTOR complex 2 to mediate many PI3K responses, including growth, metabolism, survival, and glucose homeostasis [16]. AKT promotes apoptosis resistance by phosphorylating and thereby inhibiting proapoptotic substrates and profoundly influences cellular metabolism through its direct effects on metabolic enzymes and, more indirectly, through the stimulation of mTOR complex 1 (mTORC1) activity (**Figure 1**).

The PI3K-AKT pathway is central to the integration of growth factor-derived signals and nutrient availability with anabolic metabolism, growth, and cell cycle progression in multicellular organisms. In diseases such as cancer, this pathway is reprogrammed to fuel stress

resistance and uncontrolled growth and couples growth factors and other hormonal stimuli to the metabolic and autophagy networks.

2.2. The Wnt pathway

Wnt signaling is a key pathway in embryonic development and adult homeostasis and aberrant activation of this pathway plays an important role in the development of many human cancer types [1, 2]. Indeed, aberrant Wnt signaling is a hallmark of the majority of colorectal cancers and it is implicated in maintenance of tumor-initiating cells, drug resistance, tumor progression, and metastasis.

Canonical Wnt signals are transduced through Frizzled family receptors and LRP5/LRP6 co-receptor to regulate the phosphorylation and degradation of the transcription co-activator β -catenin (**Figure 2**). Noncanonical Wnt signals are transduced independently of β -catenin through Frizzled family receptors and ROR2/RYK co-receptors to the Rho family guanosine triphosphatases, c-jun-NH₂-terminal kinase, or the Ca²⁺-dependent signaling cascades.

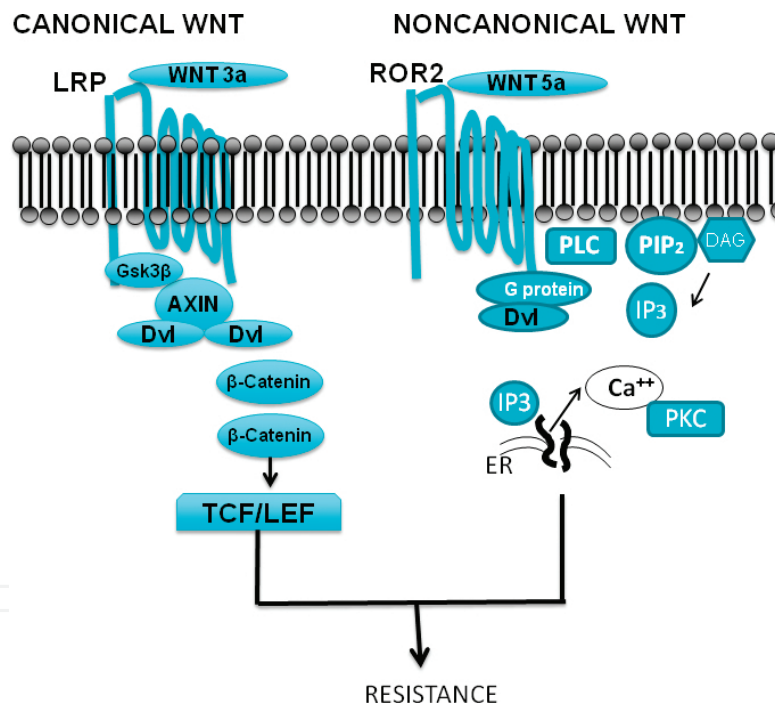


Figure 2. The canonical Wnt pathway shown controls β -catenin intracellular levels and localization. When it is activated, β -catenin is stabilized and translocated to the nucleus to bind TCF transcription factor and activate Wnt-responsive genes. Noncanonical Wnt pathway transduces signals in a β -catenin-independent manner and its activation has been associated with aggressive malignant phenotype.

In the absence of Wnt ligands, β -catenin is assembled into the so-called destruction complex assembled by adenomatous polyposis coli (APC) tumor suppressor, axin, glycogen synthase kinase-3 β (GSK-3 β), and casein kinase 1 (CK1). This complex promotes phosphorylation of β -catenin that targets it for ubiquitination and subsequent proteolysis via the proteasome [2, 3]. Upon Wnt stimulation β -catenin breakdown is inhibited, thereby causing its accumulation and

entry to the nucleus, to activate Wnt target genes [2]. In noncanonical Wnt pathways, Wnt signals are transduced independently of β -catenin. The best-studied noncanonical Wnt pathways are planar cell polarity (PCP) pathway and Ca^{2+} pathway, which play central roles in developmental morphogenesis, cell polarity, and cell migration. In Wnt/ Ca^{2+} pathway, Wnt-Frizzled (Fzd) binding activates phospholipase C (PLC) via G proteins, producing an increase of intracellular Ca^{2+} concentration and the activation of downstream effectors including protein kinase C, as it can be seen in **Figure 2** [1].

A great effort in developing selective drugs to target components of the Wnt pathway, particularly the β -catenin-dependent pathway, with anticancer activity, is underway but only a few of them have reached phase I clinical trials. In this respect, in models of KRAS-mutant colorectal cancer, it has been found by RNA-Seq data analysis for differential expression of canonical Wnt target genes that resistant cells display increased Wnt- β -catenin transcriptional activity [10], but there is also evidence, using this technique, that activation of noncanonical Wnt signaling exists in cancer-resistant cells and in different subsets of circulating tumor cells (CTCs) obtained from patients [17].

2.3. Ras-Raf-MEK signaling pathway

As mentioned before, oncogenic drivers often elicit a strong tumor dependence on the pathway that the driver controls, leading to the so-called pathway addiction. One such oncogenic driver that elicits a pathway addiction is mutant KRAS which is directly implicated in the simplified linear RAS-RAF-MEK ERK (extracellular signal-regulated kinase) signaling axis, as well as the

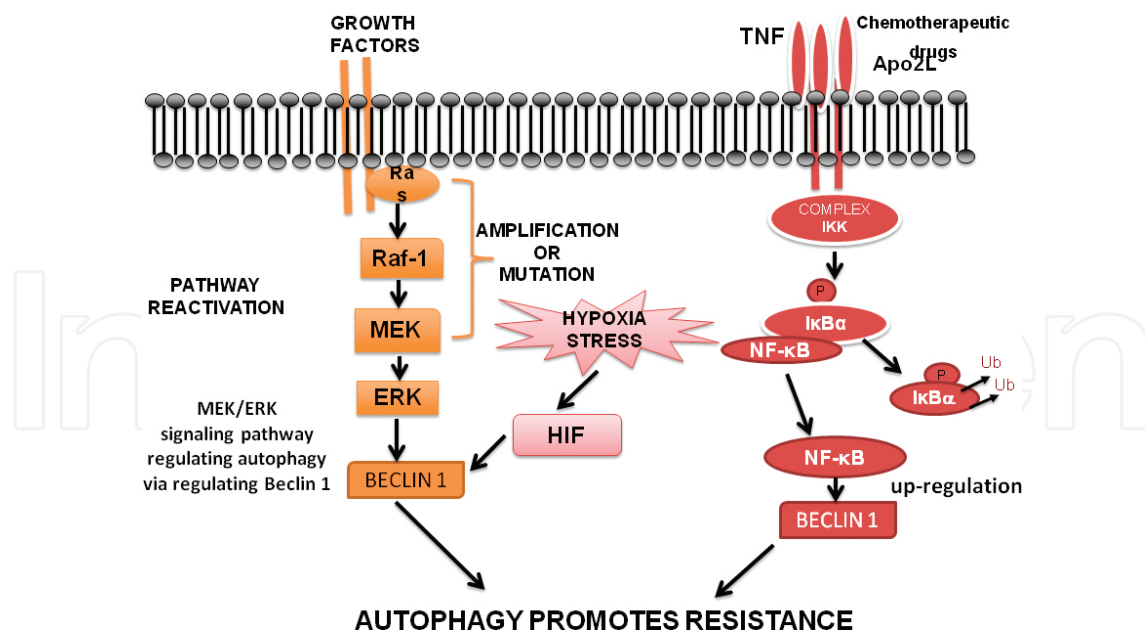


Figure 3. The Ras-Raf-MEK and NF- κ B signaling pathways promote resistance. Mutations and amplification of Ras-Raf-MEK are frequently found in colorectal cancer allowing the development of resistance to treatment via autophagy activation. NF- κ B upregulates Beclin 1 expression allowing positive regulation of autophagy. On the other hand, HIF-1 α also modulates Beclin 1. IKK can promote the autophagy in an NF- κ B-dependent manner.

PI3K-mTOR axis [10]. Mutations within RAS-Raf-Mex pathway have frequently detected in colorectal cancer, being BRAFV660E the most frequently found (**Figure 3**).

Little et al. [18] and Corcoran et al. [19] examined the mechanisms whereby colorectal cancers develop resistance. Both studies focused on the effects produced by inhibition of MEK (mitogen-activated or extracellular signal-regulated protein kinase kinase), which is a downstream effector of the oncogene BRAF or KRAS [9]. Both groups found that resistance arose through amplification of the driving oncogene (BRAF or KRAS) rather than through amplification or mutation of the targeted kinase itself (MEK).

There is also evidence that *BRAF* oncogene induces the expression of key autophagic markers, like microtubule-associated protein 1 light chain 3 (LC3) and Beclin 1 (BECN1) in colorectal tumor cells. Goulielmaki et al. [20] provided strong evidence that pretreatment with the autophagy inhibitor 3-methyl adenine (3-MA) followed by its combination with BRAFV600E targeting drug PLX4720 can synergistically sensitize resistant colorectal tumors.

2.4. LKB1/AMPK pathway

AMPK is the central metabolic sensor activated by elevated AMP/ATP ratios. LKB1 is a human tumor suppressor kinase that is a crucial upstream molecule for the activation of AMPK and hence links cell metabolism to growth control and cell polarity [21]. During nutrient and energy depletion, ATP becomes depleted while the AMP/ATP ratio rises, activating the energy-sensing kinase, LKB1, which consequently activates AMPK. The AMPK regulates mTORC1 by direct phosphorylation of the tumor suppressor tuberous sclerosis complex 2 (TSC2) to activate mTORC1 via Rheb GTPase (**Figure 1**).

2.5. Nuclear factor-kB

Aberrant activation of the nuclear factor kappa B (NF- κ B) family of dimeric transcription factors has been linked to most cellular processes in tumor evolution including inflammation, transformation, proliferation, invasion, metastasis, and chemoresistance [22]. The most common constitutively active form reported in human malignancies is the p50/RelA dimer, but other forms, such as p50/p50, p52/p52, p52/RelA, p50/c-Rel, c-Rel/c-Rel, p52/RelB, and p50/RelB, have also been identified [22]. In normal inactivated state, this transcription factor is sequestered in the cytoplasm by its inhibitor I κ B (**Figure 3**). In order to be activated, I κ B must be phosphorylated by the I κ B kinase (IKK) complex and degraded via proteasome, causing the liberation and translocation of NF- κ B into the nucleus where it modulates gene expression.

Experimental evidence demonstrates that NF- κ B can positively regulate autophagy (**Figure 3**). For instance, RelA upregulates Beclin 1 expression through direct binding to the NF- κ B-binding site on the Beclin 1 gene promoter to induce autophagy [23]. The multilevel control of autophagy by the IKK/I κ B/NF- κ B axis is also highlighted by the finding that IKK may also promote the autophagic pathway in a manner independent of NF- κ B. All these findings support the idea that NF- κ B activation promotes autophagy.

3. Metabolic stress resistance

Metabolism is the process by which cells convert relatively simple extracellular nutrients into energy and building blocks necessary for their growth and survival. In cancer cells, metabolism is dramatically altered compared with normal cells.

Metabolic stress in tumors arises from multiple factors, including cell growth and proliferation in the setting of fluctuating supplies of oxygen and nutrients. Lack of adequate blood supply leads to several stress types in tumors: hypoxia, nutrient deprivation, and the accumulation of metabolic waste products [15]. These stress types are relieved in part by the over-expression of hypoxia-inducible factors (HIFs) to release proangiogenic factors for the construction of a tumor-associated vasculature. Persistent metabolic stress in tumors provokes the rewiring of the metabolic network to accommodate the stressful tumor microenvironment [17].

Metabolic reprogramming is a hallmark of cancer cells and is used by them for growth and survival. Their metabolism is highly dependent on glycolysis instead of mitochondrial oxidative phosphorylation, regardless of oxygen availability, a process termed as Warburg effect [15]. Glycolysis alone, although relatively inefficient means to produce ATP, provides a mechanism for rapid energy generation and a source of carbon for macromolecular synthesis. In addition, the shift to glycolytic metabolism allows rapidly proliferating cells to support both energy production and biosynthesis [15]. In addition, the glycolytic pathway generates metabolites that can be efficiently diverted into pathways that support nucleotide and amino acid biosynthesis in cycling cells [15].

One interesting facet, from a chemotherapeutic point of view, is that, in a large number of cases, cells undergoing a Warburg effect exhibit a marked dependence upon glutamine, to the extent that these cells are referred to as being “glutamine addicted” [24]. Glutamine addiction arises from the need for extracellular glutamine to be consumed for anaplerotic input in the citric acid cycle, which accounts for the majority of the bioenergetic needs of normal (non-transformed) cells. Cancer cells rely heavily on glutamine as a source of carbon and nitrogen for the synthesis of ATP, proteins, lipids, nucleic acids, and the antioxidant glutathione. Expression of the Wnt target MYC proto-oncogene increases glutamine uptake by stimulating expression of the glutamine transporters. At the same time, MYC increases the levels of glutaminase 1 (GLS1), the enzyme that converts glutamine to glutamate [15, 24].

During oncogenesis, the survival signaling pathways such as PI3K, intermediate metabolism, and autophagy constitute the metabolism-signaling supernetwork reprogrammed in ways that support aberrant cell growth, proliferation, and stress resistance. Indeed, emerging evidence suggests that autophagy is an important source of amino acids that supports the stressed cell's biosynthetic and bioenergetic needs.

4. Hypoxia and drug resistance

Diminished oxygen availability (hypoxia) is a hallmark of the tumor microenvironment. A major regulator of cellular adaptation to hypoxia is the hypoxia-inducible factor (HIF) family

of transcription factors, which play key roles in many crucial aspects of cancer biology including angiogenesis, stem cell maintenance, metabolic reprogramming, resistance to apoptosis, autocrine growth factor signaling, the epithelial-mesenchymal transition (EMT) program, genetic instability, invasion, metastasis, and radiation resistance [25] (**Figure 4**). HIFs also cause autophagy establishment, particularly in RAS-driven and BRAF-driven cancers, such as most colorectal carcinomas (**Figure 4**). However, the relevance of the relation between HIFs and autophagy in drug resistance is not well understood.

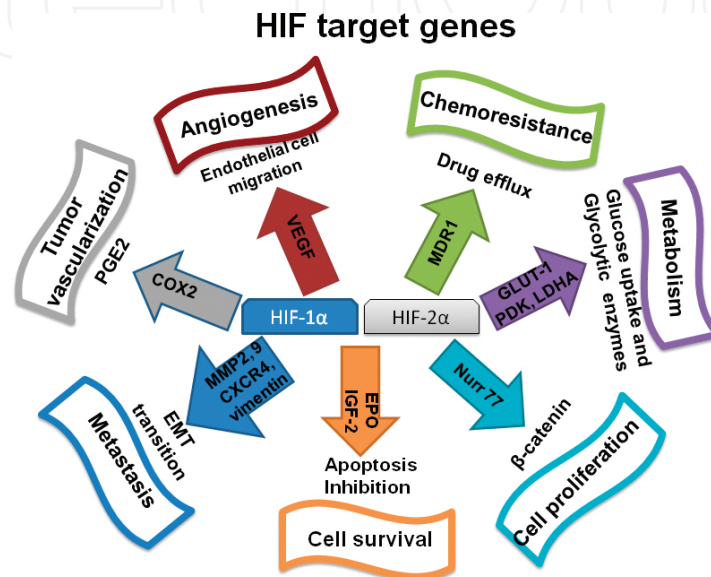


Figure 4. HIF target genes. The cellular processes regulated by HIF-1 α and HIF-2 α genes are indicated and also the target genes for each one.

HIFs are heterodimeric transcription factors consisting of an O₂-sensitive subunit HIF- α and a stable subunit HIF- β (or ARNT) that are expressed constitutively at the transcriptional and translational levels (**Figure 5**). In mammals, three HIF- α isoforms have been identified but HIF-1 α and HIF-2 α are the two best-studied members of the HIF- α family. Under normoxic conditions, the cellular stability and activity of HIF- α subunits are highly dependent on oxygen supply. Prolyl hydroxylases hydroxylate key proline residues on HIFs, which allows them to interact with the von Hippel-Lindau (pVHL) tumor suppressor, which is a component of an E3 ubiquitin ligase complex that targets HIF- α for proteasomal degradation (**Figure 5**). Hypoxic conditions stabilize HIF- α by inhibiting its hydroxylation and proteasomal degradation, making HIF- α capable to translocate to the nucleus and dimerize with ARNT activating the transcription of hypoxia-associated genes [25, 26]. Importantly, high levels of HIFs expression have also been detected in tumor cells in the absence of hypoxia (**Figure 5**) because the sustained oncogenic signaling mediated by growth factors in cancer cells can induce HIF- α expression through O₂-independent mechanisms, including increased transcription and/or translation of HIF- α mRNA [27, 28]. In this respect, we have reported that colon carcinoma cells co-express HIF-1 α and HIF-2 α under normoxic conditions, in contrast to nonmalignant colon cells, which do not express these factors under these conditions [3].

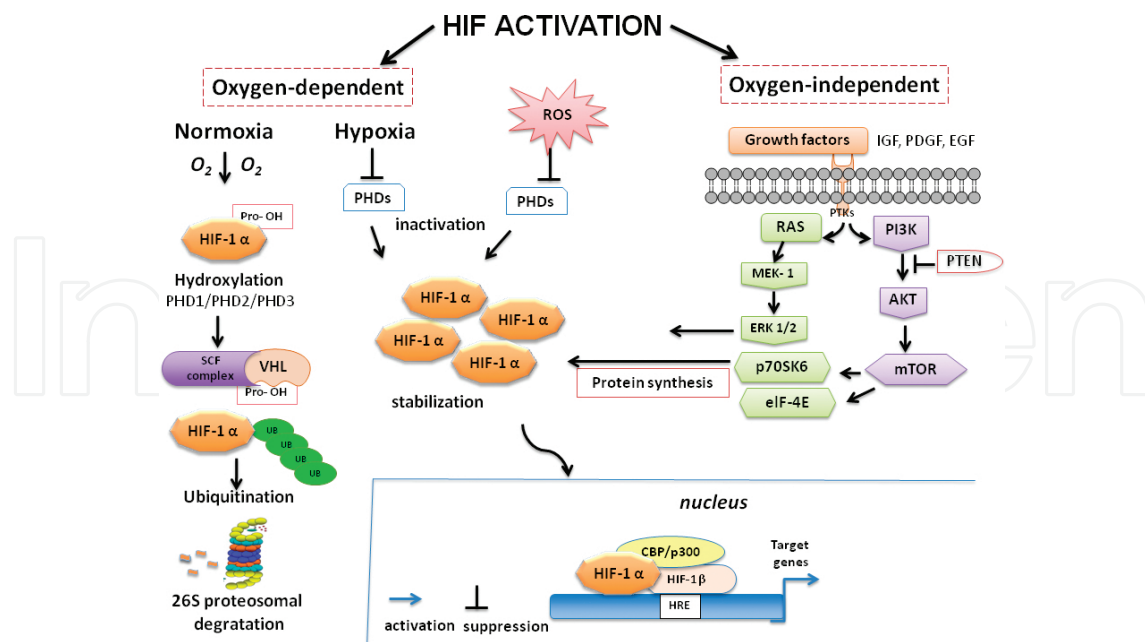


Figure 5. HIF activation. HIFs are heterodimeric transcription factors subjected to O_2 -sensitive or O_2 -independent mode of activation, as indicated in the figure. Both types of regulation converge in HIF protein stabilization, which can enter to the nucleus to bind HIF-1 β at HIF-responsive elements (HRE) to activate target genes.

Hypoxia can lead to therapeutic resistance through diverse mechanisms [29, 30]: (1) direct effects due to the requirement of some drugs and radiation of lack of oxygen in order to be maximally cytotoxic, (2) indirect effects by altering cellular metabolism to decrease drug cytotoxicity, and (3) enhanced genetic instability which in turn leads to more rapid induction of drug resistance in tumor cells [29, 30]. HIF-1 α and to a lesser extent HIF-2 α have been associated with radio- and chemotherapy failure for decades [30] and interference with HIF function holds great promise to improve future anticancer therapy.

4.1. Hypoxia-induced drug resistance through metabolic alteration

One of the possible mechanisms of resistance to anticancer therapy in hypoxic tumor is the switching of cellular metabolism from oxidative phosphorylation to aerobic glycolysis (Warburg effect), a hallmark of cancer cells. As a result, cancer cells eventually develop a system that uses cytoplasmic glycolysis to generate ATP instead of mitochondrial oxidative respiration, even in the presence of oxygen. Several reports have indicated that activation of the HIF-1 α signaling pathway under glucose deprivation induces resistance to cell death by apoptosis in human colon cancer cells and that targeting the HIF-1 α signaling pathway may provide an effective way to treat resistant cancers to conventional therapy [31].

4.2. Hypoxia-induced drug resistance through increased drug efflux

HIF-1 α upregulation has been shown to induce expression of drug efflux transporters, to alter the activity of DNA repair mechanisms, and to shift the balance between pro- and antiapoptotic factors toward cell survival [32].

Drug efflux is an important mechanism to chemoresistance in many solid tumors, including colon cancer. The multidrug resistance 1 (MDR1) gene, encoding the membrane-resident P-glycoprotein (P-gp) that belongs to a family of ATP-binding cassette (ABC) transporters, has been found to be a HIF-1 α target gene [33]. The multidrug resistance-associated protein 1 (MRP1) is another ABC transporter encoded by the *ABCC1/MRP1* gene that confers cellular resistance to a broad range of structurally and functionally chemotherapeutic agents. Recently, it has been demonstrated that MRP1 is a downstream target gene of HIF-1 α in human colon cancer LoVo cells. Genetic inhibition of HIF-1 α by siRNA and dominant-negative HIF-1 α reduced the expression of MRP1, which provides a potential novel mechanism for HIF-1 α -mediated drug resistance [34].

4.3. Hypoxia-induced drug resistance through inhibition of apoptotic pathways and induction of autophagy

Defective apoptosis and/or changes in cell cycle regulation represent pivotal causes for drug resistance [35]. It has been proposed that increased cell survival, due to a shift favoring antiapoptotic pathways, is a primary mechanism of hypoxia-induced drug resistance [36]. In the vast majority of transformed cells, HIF-1 α functions as a suppressor of apoptosis and functional interference with HIF-1 α results in enhanced cell death upon treatment with chemotherapeutic agents in tumors of different origins [26]. Through the inhibition of proapoptotic and induction of antiapoptotic genes, HIF-1 α can inhibit apoptosis and promote tumor cell survival in chemotherapy-treated cancer cells.

In colorectal cancer, the combination therapy of rapamycin, inhibitor of mTOR which regulates autophagy, and irinotecan, which is able to inhibit the accumulation of HIF-1 α , has been reported to induce massive death of colon cancer cells under hypoxic, but not normoxic conditions in vitro, and a great reduction of tumor volume in vivo [37].

Enhanced autophagy has been associated with the elevated level of HIF-1 α in several cancer types. In this regard, it has been observed that hypoxia-mediated failure of cytotoxic treatment in vitro can be conferred via HIF-1 α -dependent induction of autophagy [14], but the relevance of the intriguing relation between HIF-1 α and autophagy for drug resistance is still not known.

5. Role of autophagy in stress resistance

Autophagy is a term derived from the Greek words “auto” (self) and “phagy” (to eat) and refers to a multistep lysosomal degradation process in which a cell degrades damaged organelles and longlived proteins to maintain cellular homeostasis, particularly during exposure to stressful conditions. Three forms of autophagy have been identified based upon the mode of delivery to the lysosome, namely, macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy (autophagy), the best characterized, is a major regulated catabolic process that involves the delivery of cytoplasmic cargo sequestered inside double-membrane vesicles to the lysosome [38]. The other two forms, microautophagy and chaperone-mediated autophagy, involve a direct membrane invagination to engulf

damaged proteins and the translocation of soluble cytosolic proteins by chaperone-dependent selection across the lysosomal membrane, respectively [39]. Thirty-six genes (ATGs for AuTophagy), have been identified that are required for the autophagy process to occur. Dysregulation of autophagy, which alters the rate of protein degradation and the metabolic state of the cells, has severe consequences and is associated with several pathophysiological conditions, such as cancer.

The best-characterized regulator of autophagy is mTOR, which integrates growth factor and nutrient signals to influence protein synthesis, growth, autophagy, and ribosomal biogenesis. M-TOR occurs in two multiprotein complexes, mTORC1, containing the specific binding proteins raptor and PRAS40, and mTORC2 containing rictor and other binding partners [38]. When nutrients are available, mTORC1 phosphorylates Unc-51-like kinase (ULK1) and ATG13 to block autophagy initiation. When nutrients are scarce, mTORC1 dissociates from the ULK1 complex, initiating the autophagy process [15, 40]. The initiation of autophagy occurs with the assembly of the double-membrane phagophore, the precursor of the autophagosome. This requires the formation of a protein complex constituted by Class III phosphatidylinositol 3-kinase (PtdIns3K) and the proteins VPS34, p150, ATG14, and BECLIN 1. In tumors, autophagy is stimulated by metabolic stress (e.g., nutrient/growth factor deprivation, hypoxia, and acidosis), cellular damage, or inhibition of pro-survival signals caused by anticancer therapies [41]. Through autophagy, cancer cells utilize a highly plastic and dynamic mechanism to either repress initial steps of carcinogenesis or support the survival and growth of established tumors [42, 43].

In multicellular organisms, autophagy also clears ubiquitinated or malfunctioning aggregated proteins. This selective degradative process is mediated through the recognition of ubiquitin-tagged cargos by the autophagy receptor p62/sequestosome 1 [44]. This protein directs them to the autophagosome through concomitant binding to the LC3 (microtubule associated protein 1 light chain 3) molecules localized at the inner and outer autophagosome membranes. Autophagy is responsible for the degradation of p62, and therefore, when autophagy is inhibited, p62 accumulates in mammalian cells.

Autophagy facilitates cancer cell resistance to chemotherapy treatment, and the inhibition of autophagy may potentiate the resensitization of therapeutic-resistant cancer cells to anticancer drugs. Chemotherapy treatment conferred resistance by triggering key autophagy signaling molecules in malignant cells. For example, in response to irinotecan, pro-survival autophagy was induced by activating MAPK14/p38 signaling, which lead to drug resistance [45]. It is likely that protective autophagy in 5-Fluoracil (5-FU) resistance occurs through c-Jun N-terminal Kinase (JNK) activation [46]. Given the propensity of PI3K-mTOR inhibition to robustly induce autophagy, increased autophagic flux is a suspect contributor to the modest efficacy of PI3K inhibitors. Several recent studies have provided evidence that combined inhibition of autophagy and PI3K inhibitors or a combination therapy with a mTORC1 inhibitor (temsirolimus) and autophagy inhibitor [hydroxychloroquine (HCQ)] can sensitize cells to chemotherapeutic agents [15, 47]. Several other trials that combine HCQ with radiation therapy or chemotherapeutic agents have also been used on the basis of the accumulating

preclinical evidence supporting the notion that increased autophagy promotes therapy-induced resistance in tumor cells.

Examining the role of autophagy in RAS- and BRAF-induced transformation in colon cancer cell lines, Goulielmaki et al. [20] found that the MEK/ERK pathway can increase the protein levels of LC3, unlike the AKT/MTOR pathway, which has been shown to abolish the autophagic process. They showed that using specific autophagy inhibitors not only cancer cell proliferation rate can be reduced, but the otherwise resistant mutant BRAF colon cell lines to targeted BRAF agents, like PLX4720 (Vemurafenib), can be sensitized to apoptosis in a synergistic manner. This study proposed a promising rational combinatorial treatment using BRAF and autophagy inhibitors that will potentially provide efficient therapeutic protocols for these otherwise untreatable tumors.

6. Contribution of stem cells to resistance

The intestinal epithelium is an example of self-renewing tissue on expense of stem cells that reside at the crypt bottom. Tumor growth is sustained by a subpopulation of highly malignant cancer stem cells (CSCs) or tumor-initiating cells, which are characterized by a life-long capacity to self-renew, are multipotent, and can reversibly enter quiescent or even dormant states and resist cytotoxic drugs [48]. Successful treatment is thus dependent on the selective elimination of these highly resistant subpopulations, instead of only the main tumor mass. Over the past decade, several cell surface markers have been identified in these cell populations. CD133, Lgr5, and CD44 are the most frequently proposed stem cell markers in colorectal cancer, but their distribution differs between patients and tumor cell lines [48, 49].

CD133 (also called prominin-1) is a pentaspan transmembrane glycoprotein identified as cell surface stem cell marker that has been associated with tumorigenicity and progression of colon cancer [50], but its precise role and functions are unknown. CD44 is a transmembrane glycoprotein involved in cell-cell and cell-matrix adhesion through its affinity for hyaluronic acid. CD44 is encoded by a single gene, including 20 exons. The standard form, expressed in normal adult stem cells (referred to as CD44), consists of exons 1–5 and 15–20. Importantly, it has been demonstrated that cancer cells express different exon variants, such as CD44v6, produced by alternative splicing mRNA processing [48]. Thus, universal targeting of CD44 might be deleterious for patients and they can be avoided targeting different isoforms of CD44.

Several studies have implicated the potential contribution of a subpopulation of stem-like progenitor cells in resistance to both chemotherapeutic and targeted therapies [51]. Various groups have characterized the drug-resistant aspects of such stem-like subpopulations, including a quiescent state refractory to agents targeting rapidly dividing cells, enhanced DNA damage repair mechanisms, and decreased apoptotic machinery [52]. Recent studies have implicated a potential mechanistic link between EGFR activation and the acquisition of stem-like properties including the increase in known stem cell markers and enhanced spheroid formation [53]; however, the role of EGFR in promoting stem cell properties in CRC has not been fully characterized.

The radioresistance of cancer stem cells has been supported by several research groups in glioma and head and neck, breast, pancreatic, and colorectal cancer [48, 54]. Radiation itself has also been shown to increase the expression of AKT and CD133 and reduce the expression of CD44 in colorectal cancer cells [55]. Sahlberg et al. [48] found that cells with a CD44^{high}/CD133^{high} expression demonstrated a higher radioresistance compared to CD44^{low}/CD133^{low} cells and that different AKT isoforms have varying effects on the expression of cancer stem cell markers, which is an important consideration when targeting AKT in a clinical setting.

There is experimental evidence that hypoxia is associated with the maintenance and formation of CSCs, promoting their phenotype and tumorigenesis [56]. It has also been shown that hypoxia, by means of HIF-1 α activation, is capable of maintaining CSCs phenotype in colon cancer cells [57]. However, it has been recognized that the resident microenvironment of cancer cells, also known as a “niche,” plays an important role in the genetic instability, metastasis, and therapeutic resistance of CSCs [25]. Mao et al. [58] demonstrated that most CD133⁺ colon CSCs are located in a hypoxic niche, where oxaliplatin, rather than 5-FU, inhibits proliferation of these colon CSC cells. Recently several drugs have discovered to be selective against CSC. Examples of them are microbe-derived and plant-derived biomolecules; small inhibitors that target key signaling pathways of CSCs such as metformin, tranilast, and thioridazine; and also antibodies directed against CSC-specific cell surface molecules, such as the CD44, CD47, EpCAM, CD123, GD2, Lgr5, IGF-IR, Dll4, and FZD receptors [59].

7. Microvesicles: devices of intercellular communication

It has been demonstrated recently that challenging cancer cells with stresses that they would typically encounter during tumor progression, or as a part of a therapeutic regiment to treat or manage the disease, increase the rate of microvesicles (MVs referred also as oncosomes or exosomes), formation, and shedding by cells [5–7]. Interestingly, the noncanonical Wnt signaling pathways PCP and Wnt/Ca²⁺ appear to be involved in the regulation of MV biogenesis and budding in human cancer cells, since one of their downstream effectors, the Rho subfamily GTPase, that induce cell spreading and migration, has been demonstrated to promote the rearrangements of the actin cytoskeleton to stimulate MV budding [60, 61].

MVs generally range in size from 0.1 to 2 μ m in diameter. In addition to containing conventional paracrine signaling molecules, such as growth factors and pro-inflammatory cytokines, MVs also contain membrane-associated, cytosolic, and nuclear molecules not normally released by normal cells such as metabolic enzymes, metalloproteases, molecular chaperones, and miRNAs and RNA transcripts [6, 7]. The uptake of MVs by cells has the potential to protect them from a variety of apoptotic challenges by up-regulating the expression and/or activation of proteins that work to counter the actions of cell death machinery. That is why recent findings suggesting that MVs can promote cell survival and contribute to drug resistance are possibly of significant value.

In addition, because cancer cell-derived MVs often contain oncogenic proteins that reflect their cell of origin, and their abundance and protein concentration correlate with the tumor grade/aggressiveness, they have been converted in the focus for searching cancer biomarkers and/or for monitoring tumor progression.

8. Concluding remarks

Mammalian cells coordinate cell metabolism and growth with environmentally induced stress. In cancer cells, survival signaling cascades, metabolism, and autophagy are corrupted and work in a highly integrated network to cope with stressful conditions such as hypoxia, limited nutrients, and drugs, in order to maintain viability and proliferative activity.

Understanding the signaling networks that contribute to cancer cell survival and how the changes in those networks allow cells to adapt in the presence of drugs that target key components implicated in cancer cell survival and proliferation is the challenge. Thus, we are hopeful that a better understanding of the signaling pathways involved and the development of strategies to inhibit autophagy and/or hypoxia represent a new approach to enhance the efficacy of cancer therapy to overcome therapeutic resistance in cancer cells.

Author details

Dora M. Velázquez, María Cristina Castañeda-Patlán and Martha Robles-Flores*

*Address all correspondence to: rmartha@unam.mx

Department of Biochemistry, Faculty of Medicine, National Autonomous University of Mexico (UNAM), Mexico City, Mexico

References

- [1] Behrens J. Everything you would like to know about Wnt signaling. *Sci Signal* 2013, 6:pe17
- [2] Clevers H, Nusse R. Wnt/ β -catenin signaling and disease. *Cell* 2012, 149:1182–1205.
- [3] Santoyo-Ramos P, Likhatcheva M, Castañeda-Patlán MC, García-Zepeda E, Robles-Flores M. Hypoxia-inducible factors modulate the stemness and malignancy of colon cancer cells by playing opposite roles in canonical Wnt signaling. *PLoS One* 2014, 9(11):e112580.

- [4] Xie X, White EP, Mehnert JM. Coordinate autophagy and mTOR pathway inhibition enhances cell death in melanoma. *PLoS One* 2013; 8 (1): e55096
- [5] Antonyak MA, Li B, Boroughs LK, et al. Cancer cell-derived microvesicles induce transformation by transferring tissue transglutaminase and fibronectin to recipient cells. *Proc Natl Acad Sci U S A* 2011, 108:4852–4857
- [6] Peinado H, Aleckovic M, Lavotshkin S, et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat Med* 2012, 18:883–891.
- [7] D'Souza-Schorey C, Clancy JW. Tumor-derived microvesicles: shedding light on novel microenvironment modulators and prospective cancer biomarkers. *Genes Dev* 2012, 26:1287–1299.
- [8] Ono M, Kosaka N, Tominaga N, Yoshida Y, Tsuda H, Tamura K, Ochiya T. Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells. *Sci Signal* 2014, 7(332):ra63. doi: 10.1126/scisignal.2005231.
- [9] Adler EM, Gough NR. Focus issue: rendering resistance futile. *Sci Signal* 2011, 4(166); eg3.
- [10] Belmont PJ, Jiang P, McKee TD, Xie T, Isaacson J, Baryla NE, Roper J, Sinnamon MJ, Lee NV, Kan JLC, Guicherit O, Wouters VG, O'Brien CA, Shields D, Olson P, Van Arsdale T, Weinrich SL, Rejto P, Christensen JG, Fantin VR, Hung KE, Martin ES. Resistance to dual blockade of the kinases PI3K and mTOR in KRAS-mutant colorectal cancer models results in combined sensitivity to inhibition of the receptor tyrosine kinase EGFR. *Sci Signal* 2014, 7(351):ra107.
- [11] Crea F, Nobili S, Paolicchi E, Perrone G, Napoli C, Landini I, Danesi R, Mini E. Epigenetics and chemoresistance in colorectal cancer: an opportunity for treatment tailoring and novel therapeutic strategies. *Drug Resist Updat* 2011, 14:280–296
- [12] Yang AD, Fan F, Camp ER, van Buren G, Liu W, Somcio R, Gray MJ, Cheng H, Hoff PM, Ellis LM. Chronic oxaliplatin resistance induces epithelial-to-mesenchymal transition in colorectal cancer cell lines. *Clin Cancer Res* 2006, 12:4147–4153.
- [13] Singh A, Settleman J, EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. *Oncogene* 2010, 29:4741–4751.
- [14] Martz CA, Ottina KA, Singleton KR, Jasper JS, Wardell SE, Peraza-Penton A, Anderson GR, Winter PS, Wang T, Alley HM, Kwong LN, Cooper ZA, Tetzlaff M, Chen PL, Rathmell JC, Flaherty KT, Wargo JA, McDonnell DP, Sabatini DM, Wood KC. Systematic identification of signaling pathways with potential to confer anticancer drug resistance. *Sci Signal* 2014, 7(357); ra121.

- [15] Shanware NP, Bray K, Abraham RT. The PI3K, metabolic, and autophagy networks: interactive partners in cellular health and disease. *Annu Rev Pharmacol Toxicol* 2013, 53:89–106
- [16] Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell* 2007, 129:1261–1274
- [17] Miyamoto DT, Zheng Y, Wittner BS, Lee RJ, Zhu H, Broderick KT, Desai R, Fox DB, Brannigan BW, Trautwein J, Arora KS, Desai N, Dahl DM, Sequist LV, Smith MR, Kapur R, Wu CH, Shioda K, Ramaswamy S, Ting DT, Toner M, Maheswaran S, Haber DA. RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. *Sci Signal* 2015, 349(6254):1351–1356.
- [18] Little AS, Balmanno K, Sale MJ, Newman S, Dry JR, Hampson M, Edwards PAW, Smith PD, Cook SJ. Amplification of the driving oncogene, KRAS or BRAF, underpins acquired resistance to MEK1/2 inhibitors in colorectal cancer cells. *Sci Signal* 2011, 4:ra17.
- [19] Corcoran RB, Dias-Santagata D, Bergethon K, Iafrate AJ, Settleman J, Engelman JA. BRAF gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the BRAF V600E mutation. *Sci Signal* 2010, 3:ra84.
- [20] Goulielmaki M, Koustas E, Moysidou E, Vlassi M, Sasazuki T, Shirasawa S, Zografos G, Oikonomou E, Pintzas A. BRAF associated autophagy exploitation: BRAF and autophagy inhibitors synergise to efficiently overcome resistance of BRAF mutant colorectal cancer cells. *Oncotarget* 2016, 7(8):9188–9221.
- [21] Shackelford DB, Shaw RJ. The LKB1–AMPK pathway: metabolism and growth control in tumour suppression. *Nat Rev Cancer* 2009, 9:563–575.
- [22] Chen F, Castranova V. Nuclear factor-kappaB, an unappreciated tumor suppressor. *Cancer Res* 2007, 67:11093–11098.
- [23] Copetti T, Bertoli C, Dalla E, Demarchi F, Schneider C. p65/RelA modulates BECN1 transcription and autophagy. *Mol Cell Biol* 2009, 29:2594–2608.
- [24] Katt WP, Cerione RA. Glutaminase regulation in cancer cells: a druggable chain of events. *Drug Discov Today* 2014, 19(4):450–457.
- [25] Semenza, G.L. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene* 2010, 29:625–634.
- [26] Rohwer N, Cramer T. Hypoxia-mediated drug resistance: novel insights on the functional interaction of HIFs and cell death pathways. *Drug Resist Updat* 2011, 14:191–201.
- [27] Koh MY, Powis G. HIF: the new player in oxygen-independent HIF-1 α degradation. *Cell Cycle* 2009, 8: 1359–1366.

- [28] Richard DE, Berra E, Gothié E, Roux D, Pouyssegur J. P42/P44 mitogen activated protein kinases phosphorylate hypoxia-inducible factor 1 alpha and enhance the transcriptional activity of HIF-1. *J Biol Chem* 1999, 274:32631–32637.
- [29] Teicher, B.A. Hypoxia and drug resistance. *Cancer Metastasis Rev* 1994, 13:139–168.
- [30] Mimeault M, Batra S. Hypoxia-inducing factors as master regulators of stemness properties and altered metabolism of cancer- and metastasis-initiating cells. *J Cell Mol Med* 2013, 17:30–54.
- [31] Nishimoto A, Kugimiya N, Hosoyama T, Enoki T, Li T-S, Hamano K. HIF-1 α activation under glucose deprivation plays a central role in the acquisition of anti-apoptosis in human colon cancer cells. *Int J Oncol* 2014, 44:2077–2084.
- [32] Semenza GL. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. *J Clin Invest* 2013, 123:3664–3671.
- [33] Comerford KM, Wallace TJ, Karhausen J, Louis NA, Montalto MC, Colgan SP. Hypoxia-inducible factor-1-dependent regulation of the multidrug resistance (MDR1) gene. *Cancer Res* 2002, 62:3387–3394.
- [34] Lv Y, Zhao S, Han J, Zheng L, Yang Z, Zhao L. Hypoxia-inducible factor-1 α induces multidrug resistance protein in colon cancer. *Onco Targets Ther* 2015, 5:1941–1948.
- [35] Brown JM, Attardi LD. The role of apoptosis in cancer development and treatment response. *Nat Rev Cancer* 2005, 5:231–237.
- [36] Erler, J.T., Cawthorne, C.J., Williams, K.J., Koritzinsky, M., Wouters, B.G., Wilson, C. Hypoxia-mediated down-regulation of Bid and Bax in tumors occurs via hypoxia-inducible factor 1-dependent and -independent mechanisms and contributes to drug resistance. *Mol Cell Biol* 2004, 24:2875–2889.
- [37] Pencreach E, Guerin E, Nicolet C, Lelong-Rebel I, Voegeli AC, Oudet P, Larsen AK, Gaub MP and Guenot D. Marked activity of irinotecan and rapamycin combination toward colon cancer cells in vivo and in vitro is mediated through cooperative modulation of the mammalian target of rapamycin/hypoxia-inducible factor-1 α axis. *Clin Cancer Res* 2009, 15:1297–1307.
- [38] Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. *Cell* 2011, 147:728–741.
- [39] Klionsky DJ. The molecular machinery of autophagy: unanswered questions. *J Cell Sci* 2005, 118:7–18.
- [40] Xie CM, Liu XY, Sham KW, Lai JM, Cheng CH. Silencing of EEF2K (eukaryotic elongation factor-2 kinase) reveals AMPK–ULK1-dependent autophagy in colon cancer cells. *Autophagy* 2014, 10:1495–1508.
- [41] Simonsen A, Tooze SA. Coordination of membrane events during autophagy by multiple class III PI3-kinase complexes. *J Cell Biol* 2009, 186:773–782.

- [42] Geng J, Klionsky DJ. The Atg8 and Atg12 ubiquitin-like conjugation systems in macroautophagy. *EMBO Rep* 2008, 9:859–864.
- [43] Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, et al. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosomal membranes after processing. *EMBO J* 2000, 19:5720–5728.
- [44] Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem* 2007, 282:24131–24145.
- [45] Paillas S, Causse A, Marzi L, de Medina P, Poirot M, Denis V, et al. MAPK14/p38 confers irinotecan resistance to TP53-defective cells by inducing survival autophagy. *Autophagy* 2012, 8:1098–1112
- [46] Sui X, Kong N, Wang X, Fang Y, Hu X, Xu Y, et al. JNK confers 5-fluorouracil resistance in p53-deficient and mutant p53-expressing colon cancer cells by inducing survival autophagy. *Sci Rep* 2014, 4:4694.
- [47] Li J, Hou N, Faried A, Tsutsumi S, Kuwano H. Inhibition of autophagy augments 5-fluorouracil chemotherapy in human colon cancer in vitro and in vivo model. *Eur J Cancer* 2010, 46:1900–1909.
- [48] Sahlberg SH, Spiegelberg D, Glimelius B, Stenerlo B, Nestor M. Evaluation of cancer stem cell markers CD133, CD44, CD24: association with AKT isoforms and radiation resistance in colon cancer cells. *PLoS One* 2014, 9(4):e94621
- [49] Chu P, Clanton DJ, Snipas TS, Lee J, Mitchell E, et al. Characterization of a subpopulation of colon cancer cells with stem cell-like properties. *Int J Cancer* 2009, 124:1312–1321.
- [50] Horst D, Scheel SK, Liebmann S, Neumann J, Maatz S, et al. The cancer stem cell marker CD133 has high prognostic impact but unknown functional relevance for the metastasis of human colon cancer. *J Pathol* 2009, 219:427–434.
- [51] Dallas NA, Xia L, Fan F, Gray MJ, Gaur P, van Buren Jr.G, Samuel S, Kim MP, Lim SJ, Ellis LM. Chemoresistant colorectal cancer cells, the cancer stem cell phenotype, and increased sensitivity to insulin-like growth factor-I receptor inhibition. *Cancer Res* 2009, 69:1951–1957.
- [52] Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat Rev Cancer* 2005, 5:275–284.
- [53] Abhold EL, Kiang A, Rahimy E, Kuo SZ, Wang-Rodriguez J, Lopez JP, Blair KJ, Yu MA, Haas M, Brumund KT, Altuna X, Patel A, Weisman RA, Ongkeko WM. EGFR kinase promotes acquisition of stem cell-like properties: a potential therapeutic target in head and neck squamous cell carcinoma stem cells. *PLoS One* 2012, 7:e32459.

- [54] Saigusa S, Tanaka K, Toiyama Y, Yokoe T, Okugawa Y, et al. Immunohistochemical features of CD133 expression: association with resistance to chemoradiotherapy in rectal cancer. *Oncol Rep* 2010, 24:345–350.
- [55] Kawamoto A, Tanaka K, Saigusa S, Toiyama Y, Morimoto Y, et al. Clinical significance of radiation-induced CD133 expression in residual rectal cancer cells after chemoradiotherapy. *Exp Ther Med* 2012, 3:403–409.
- [56] Mohyeldin A, Garzon-Muvdi T, Quinones-Hinojosa A. Oxygen in stem cell biology: a critical component of the stem cell niche. *Cell Stem Cell* 2010, 7:150–161.
- [57] Yeung TM, Gandhi SC, Bodmer WF. Hypoxia and lineage specification of cell line-derived colorectal cancer stem cells. *Proc Natl Acad Sci U S A* 2011, 108: 4382–4387.
- [58] Mao Q, Zhang Y, Fu X, Xue J, Guo W, Meng M, Zhou Z, Mo X, and Lu Y. A tumor hypoxic niche protects human colon cancer stem cells from chemotherapy. *J Cancer Res Clin Oncol* 2013, 139:211–222
- [59] Garza-Treviño EN, Said-Fernández SL, Martínez-Rodríguez HG. Understanding the colon cancer stem cells and perspectives on treatment. *Cancer Cell Int* 2015, 15:2. doi: 10.1186/s12935-015-0163-7.
- [60] Antonyak MA, Wilson KF, Cerione RA. R(h)oads to microvesicles. *Small GTPases* 2012, 3:219–224.
- [61] Li B, Antonyak MA, Zhang J et al. RhoA triggers a specific signaling pathway that generates transforming microvesicles in cancer cells. *Oncogene* 2012, 31:4740-4749.