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Review

mTOR Signaling Confers Resistance to Targeted Cancer Drugs

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Cancer is a complex disease and a leading cause of death worldwide. Extensive research over decades has led to the development of therapies that target cancer-specific signaling pathways. However, the clinical benefits of such drugs are at best transient due to tumors displaying intrinsic or adaptive resistance. The underlying compensatory pathways that allow cancer cells to circumvent a drug blockade are poorly understood. We review here recent studies suggesting that mammalian TOR (mTOR) signaling is a major compensatory pathway conferring resistance to many cancer drugs. mTOR-mediated resistance can be cell-autonomous or non-cell-autonomous. These findings suggest that mTOR signaling should be monitored routinely in tumors and that an mTOR inhibitor should be considered as a co-therapy.

Resistance Mechanisms Limit the Success of Cancer Therapeutics

Over recent decades many small molecules have been developed to specifically target oncogenic pathways. However, with few exceptions, these drugs as a single agent have not led to a cure. The limited success of targeted drugs is due to tumors displaying resistance. Two modes of cancer drug resistance exist, intrinsic and adaptive (also referred to as evasive or acquired). Intrinsic resistance is non-responsiveness to a therapy, whereas adaptive resistance is defined as responsiveness followed by relapse. Intrinsic resistance is generally the result of a tumor widely containing a pre-existing mutation that confers resistance in a cell-autonomous manner. Adaptive resistance can be similarly inherent to the cancer cell, but with the genetic or epigenetic change arising upon treatment rather than pre-existing. Importantly, adaptive resistance can also be non-inherent (i.e., non-cell-autonomous) in which resistance relies on the tumor microenvironment. This latter mechanism involving the microenvironment can be viewed as a 'physiological' stress response in which cancer cells are supported by neighboring cells. Understanding the factors that confer intrinsic or adaptive resistance is important for patient stratification and the rational design of combination therapies. Recent studies suggest that sustained mTOR signaling, in cancer cells or cells of the microenvironment, confers resistance to various primary targeted cancer therapies. Thus, mTOR signaling appears to be a major compensatory pathway conferring resistance to targeted therapies.

The mTOR Signaling Pathway

Growth and proliferation are highly regulated. The evolutionarily conserved serine/threonine kinase target of rapamycin (TOR) integrates various stimuli to control the metabolic pathways that drive cell growth and proliferation (Figure 1). TOR forms two structurally and functionally distinct multiprotein complexes termed TOR complex 1 (TORC1) and TORC2 (reviewed in [1,2]). In mammals, mTORC1 contains mTOR, mammalian lethal with sec-13 protein 8 (mLST8), and regulatory associated protein of mammalian target of rapamycin (RAPTOR). mTORC1 is activated by growth factors, nutrients, and cellular energy (reviewed in [3,4]), and is acutely

Trends

The clinical benefit of targeted cancer drugs is limited owing to intrinsic or adaptive resistance. Mechanisms of resistance can be cancer cell-autonomous or non cell-autonomous.

Drugs can alter the tumor microenvironment, resulting in dynamic rewiring of signaling circuits and resistance in neighboring cancer cells.

mTOR signaling is a major compensatory pathway allowing cancer cells to escape the effects of targeted drugs.

mTOR inhibitors should be considered as a co-therapy to prevent resistance to other targeted cancer drugs.

Phosphoproteomics is a novel approach for elucidating mechanisms of resistance to targeted therapies.

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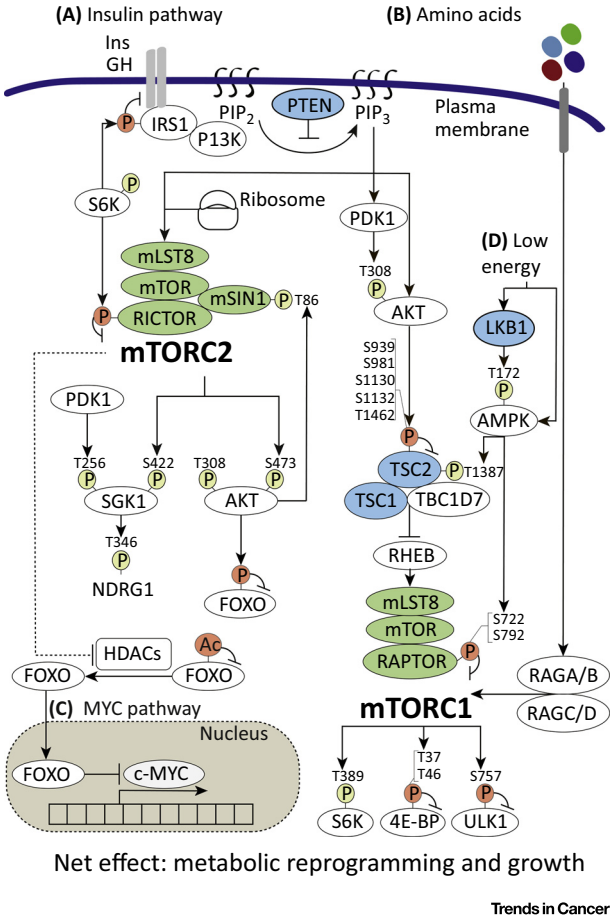

Figure 1. mTOR Signaling Network. mTOR signaling promotes cancer cell growth, survival, and proliferation. (A) Growth factors such as insulin (Ins) stimulate PI3K to convert phosphatidylinositol-4,5-bisphosphate (PIP_2) to phosphatidylinositol-3,4,5-trisphosphate (PIP_3). PIP_3 stimulates PDK1 to phosphorylate (P) AKT at T308. AKT phosphorylates TSC2 on multiple sites, thereby inhibiting its GAP activity toward RHEB. GTP-loaded RHEB binds to and activates mammalian TOR complex 1 (mTORC1). Growth factors also activate mTORC2 in a PI3K- and ribosome-dependent manner. mTORC2 phosphorylates and activates the AGC kinase family members SGK1 and AKT. mTORC2 phosphorylates AKT at Ser473. mTORC2 is not upstream of mTORC1 because AKT Ser473 phosphorylation is not required for mTORC1 activation. (B) Amino acids stimulate mTORC1 by promoting the conversion of RAS-related GTP-binding protein (RAG) heterodimers to the active conformation, in which RAGA or RAGB is loaded with GTP, and RAGC or RAGD is loaded with GDP. Active RAG heterodimer recruits mTORC1 to the surface of the lysosome where mTORC1 encounters its direct activator RHEB. (C) c-MYC, whose expression is repressed by FOXO, mediates cancer cell metabolic reprogramming. In an AKT-independent manner, mTORC2 inhibits class II HDACs, thereby increasing FOXO acetylation (Ac). Ac-FOXO is retained in the cytoplasm, unable to inhibit c-MYC expression. mTORC2 also inhibits FOXO via AKT. (D) In response to low energy (high AMP/ATP ratio), AMP-activated protein kinase (AMPK) inhibits mTORC1 activity by phosphorylating RAPTOR at S792 and S722, and by phosphorylating TSC2. The tumor-suppressor liver kinase B1 (LKB1) activates AMPKα by phosphorylating T172 in the activation loop. Blue-colored proteins are tumor-suppressors that inhibit mTOR activity. Phosphorylation depicted in green is an activation signal and phosphorylation depicted in red is an inhibitory signal. Abbreviations: Ac, acetylation; GH, growth hormone; NDRG1, N-MYC downstream-regulated gene 1.

inhibited by the macrolide rapamycin. Rapamycin (and its analogs known as rapalogs) binds to the cytoplasmic protein FKBP12 (FK506-binding protein 12), and the FKBP12–rapamycin complex then binds to the FRB (FK506-binding protein/rapamycin-binding) domain in mTOR of mTORC1 [5]. Several mTOR inhibitors are approved or in clinical trials for cancer therapy (reviewed in [5–7]). mTORC2 is not acutely inhibited by rapamycin, presumably because the FRB domain in mTOR in mTORC2 is masked [8]. Growth factors and cellular energy stimulate mTORC1 via inhibition of the heterotrimeric protein complex consisting of tuberous sclerosis

complex 1 (TSC1), TSC2, and TRE2–BUB2–CDC16 domain family member 7 (TBC1D7) [9–14], hereafter referred to as the TSC complex. Insulin (or other growth factors) bind to receptor tyrosine kinases (RTKs) to activate phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K). PI3K phosphorylates the inositol ring of the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP_2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP_3) [15]. PIP_3 recruits phosphoinositide-dependent kinase 1 (PDK1) and AKT to the plasma membrane [16]. PDK1 phosphorylates Thr308 in the activation loop of AKT and thereby activates AKT [17]. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) converts PIP_3 to PIP_2, counteracting the activity of PI3K. AKT phosphorylates TSC2, thereby inducing lysosomal release and inhibition of the TSC complex [10,11,18]. The TSC complex is a GTPase-activating protein (GAP) for the lysosomal GTP-binding protein RAS homolog enriched in brain (RHEB). GTP-loaded RHEB interacts with the mTOR catalytic domain and activates mTORC1 [19]. mTORC1 promotes anabolic processes such as protein, lipid, and nucleotide biosynthesis, and inhibits catabolic processes such as autophagy. Notable downstream targets of mTORC1 are ribosomal protein S6 kinase (S6K), eukaryotic translation initiation factor 4E binding proteins (4E-BPs), and the autophagy activating Unc-51-like kinase 1 (ULK1) (reviewed in [20–22]) (Figure 1). mTORC2 contains mTOR, mLST8, mammalian stress-activated mitogen-activated protein kinase (MAPK)-interacting protein 1 (mSIN1), and rapamycin-insensitive companion of mTOR (RICTOR). Growth factors activate mTORC2 by promoting association of mTORC2 with ribosomes in a PI3K-dependent manner [23]. PIP_3 interacts with the PH domain of mSIN1 to trigger mTORC2 activation [24]. mTORC2 regulates several cellular processes via activation of the AGC kinase family members AKT, protein kinase C (PKC), and serum/glucocorticoid-regulated kinase (SGK) (reviewed in [25,26]). mTORC2 phosphorylates Ser473 in AKT. In a positive feedback loop, AKT phosphorylates mSIN1-Thr86 in mTORC2 [27]. In a negative feedback loop, mTORC1 via S6K phosphorylates and inhibits the insulin receptor substrate 1 (IRS-1), thereby dampening PI3K signaling [28–30] (Figure 1). mTORC1 and mTORC2 are frequently activated in human cancers. Genetically engineered mouse models with ectopic activation of mTORC1 or mTORC2 develop cancer [31–34]. mTOR, often in the context of positive and negative feedback loops, is a node for convergence and crosstalk of several oncogenic pathways (Figures 1,2) [30,35–37].

mTOR Signaling in Cell-Autonomous Resistance

Extracellular signal-regulated kinase (ERK) is a MAPK and the major effector of the GTPase Kirsten rat sarcoma viral oncogene homolog (KRAS) (Figure 2). Ligand-mediated activation of RTKs triggers GTP loading of KRAS, which then recruits the kinase BRAF to the plasma membrane for activation [38]. BRAF phosphorylates and activates the MAPK kinase MEK. MEK activates ERK that in turn phosphorylates cytoplasmic signaling proteins, including p90 ribosomal S6 kinase (RSK). ERK and RSK phosphorylate and inhibit TSC2, leading to activation of mTORC1. Furthermore, it has been suggested that RSK phosphorylates several sites in RAPTOR to enhance mTORC1 activity [39]. Finally, ERK and mTORC1 provide distinct activating inputs to eukaryotic translation initiation factor 4E (eIF4E), thereby promoting cap-dependent mRNA translation [40]. Thus, mTOR and ERK signaling are functionally related.

The ERK kinase network is constitutively active in about 40% of human melanomas [41] (Figure 2). Loss of the tumor-suppressor PTEN, which leads to activation of mTOR signaling, confers poor response to BRAF inhibitors in melanoma patients [42–44]. Indeed, these patients define a distinct subset of melanoma that is resistant to BRAF inhibitors. Melanoma cell lines and human patient samples that exhibit resistance to BRAF, MEK, or ERK inhibitors display enhanced S6-S235/236 and S240/244 [45,46] or AKT-Ser473 phosphorylation [47–49], readouts of mTORC1 and mTORC2, respectively. Thus, PI3K–mTOR signaling appears to compensate for loss of ERK signaling and thereby confers resistance to BRAF-MEK-ERK inhibitors. mTOR may compensate by substituting for ERK signaling in phosphorylating particular substrates [50].

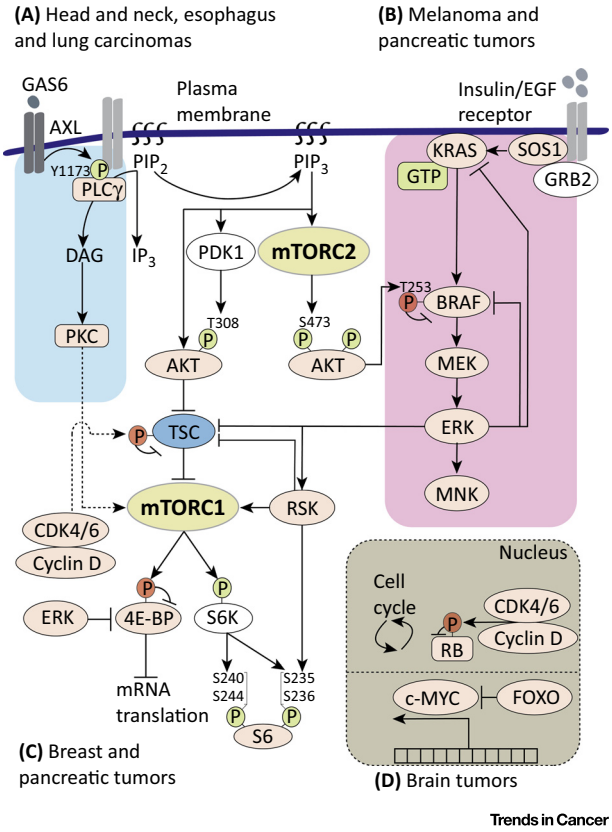

Figure 2. mTOR Signaling and Resistance to Targeted Drugs. (A) AXL is a RTK that is activated through ligand (GAS6)-dependent or -independent dimerization. In head and neck, and esophageal squamous cell carcinomas dimerization of AXL and EGFR contributes to drug resistance via activation of mTORC1. AXL phosphorylates EGFR at Y1173 that in turn serves as a docking site for phospholipase Cγ (PLCγ). PLCγ at the plasma membrane cleaves PIP_2 to produce the second messengers diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). DAG activates members of the serine/threonine protein kinase C (PKC) family. By an unknown mechanism, PKC activates mTORC1, thereby promoting resistance to several drugs. (B) The mTOR and ERK pathways respond to extracellular and intracellular cues to control cell survival, proliferation, and metabolism. SOS1 is a guanine nucleotide exchange factor for the GTPase KRAS. GTP-loaded KRAS recruits BRAF kinase to the plasma membrane for activation. BRAF phosphorylates and activates MEK. MEK activates ERK that phosphorylates cytoplasmic signaling proteins, including RSK and MNK. ERK and RSK phosphorylate and inhibit TSC2, thereby activating mTORC1. RSK phosphorylates RAPTOR on several sites to enhance mTORC1 activity. RSK also phosphorylates ribosomal protein S6 at the Ser235 and Ser236. ERK and mTORC1 provide distinct and complementary inputs to eIF4E, thereby promoting mRNA translation (not shown). The ERK pathway is activated in melanoma and pancreatic tumors. In these tumors, targeted inhibition of the ERK pathway promotes both mTORC1 and mTORC2 activation, and thereby resistance to the ERK pathway inhibitors. (C) The cyclin D–cyclin-dependent kinase (CDK) 4/6–retinoblastoma (RB) pathway regulates cell-cycle progression. Unphosphorylated RB binds to and inhibits E2F transcription factors. CDK4/6–cyclin D phosphorylates the tumor-suppressor RB that dissociates from E2F, allowing cell-cycle progression. Resistance to CDK4/6 inhibitors is associated with increased mTORC1 activity in breast and pancreatic cancers. Although the connection between CDK4/6–cyclin D and mTORC1 signaling is poorly understood, mTORC1 activation limits the killing effect of cell-cycle inhibitors. (D) Brain tumor cells containing an EGFRvIII amplification exhibit enhanced mTORC2 activity. mTORC2 mediates metabolic reprogramming and resistance to targeted drugs by increasing expression of c-MYC. Abbreviations: GRB2, growth factor receptor-bound protein 2; RTK, receptor tyrosine kinase; SOS, son of sevenless homolog 1.

PI3K activating mutations are common in various human cancers [51]. In breast cancer cells containing a *PIK3CA* mutation, resistance to the PI3K inhibitor BLY719 correlates with S6 hyperphosphorylation, and mTOR inhibition restores sensitivity to BLY719 [52]. Similarly, mTOR signaling confers resistance to PI3K inhibitors in thyroid tumor cells [53]. Thus, mTOR activation confers resistance to PI3K inhibitors.

The transcription factor c-MYC promotes tumor progression and metabolic adaptation [54] (Figure 2). mTORC2-dependent c-MYC overexpression, and thereby enhanced aerobic glycolysis, confers resistance to a PI3K inhibitor in glioblastoma multiforme (GBM) [47,55]. Thus, mTOR appears to confer resistance to PI3K inhibitors also via upregulation of c-MYC.

The cyclin-dependent kinase (CDK) 4/6–retinoblastoma (RB) pathway regulates cell-cycle progression [56] and is implicated in various cancers [56–58] (Figure 2). Unphosphorylated RB binds and represses E2 family (E2F) transcription factors. CDK4/6 in association with cyclin D1 phosphorylates the tumor-suppressor RB that in turn dissociates from E2F, allowing cell-cycle progression. Resistance to CDK4/6 inhibitors is associated with increased mTORC1 activity in cell lines [59] as well as in mouse models of breast [60] and pancreatic [61] cancers. Although the connection between CDK4/6–cyclin D and mTORC1 signaling is poorly understood, these studies indicate that mTORC1 activation limits the killing effect of cell-cycle inhibitors. Interestingly, phosphorylated RB appears to interact directly with mSIN1 to inhibit mTORC2 [62], suggesting that mTORC2 activation may occur in response to a CDK4/6 inhibitor and confer resistance to the drug. Thus, an ATP competitive pan-mTOR inhibitor that targets both mTORC1 and mTORC2 could be considered as a co-therapy with a CDK4/6 inhibitor.

AXL is a member of the TAM (TYRO, AXL, and MER) family of receptor tyrosine kinases (reviewed in [63,64]) (Figure 2). AXL is activated in many ways including homodimerization or heterodimerization with a non-TAM receptor [65]. AXL activation is associated with acquired resistance to PI3K, RTK, BRAF, and MEK inhibitors [66]. In head and neck, and esophageal squamous cell carcinoma cells treated with a PI3K inhibitor, AXL dimerizes with and phosphorylates EGFR. Phosphorylated EGFR-Y1173 is a docking site for phospholipase Cγ (PLCγ). PLCγ, via the second messenger diacylglycerol (DAG), activates the serine/threonine protein kinase C (PKC). Via an unknown mechanism, PKC activates mTORC1 [67], thereby conferring resistance to the PI3K inhibitor [68] (Figure 2). In cell lines and human lung tumor samples, AXL activation is associated with resistance to the EGFR inhibitors erlotinib [69] and gefitinib [70], respectively. Given the above study demonstrating that AXL can activate EGFR and ultimately mTORC1 to confer resistance to a PI3K inhibitor, AXL-mediated resistance to EGFR inhibitors is possibly also via mTORC1.

The above studies indicate that activation of the mTOR pathway can confer resistance to various targeted therapies. This underscores the complex interplay between mTOR and other major oncogenic pathways, and how such interplay can be exploited for resistance. These studies also suggest that patients should be routinely monitored for mTOR activity, and a co-therapy with an mTOR inhibitor should be considered. Clearly, the clinical application of combination therapies should be evaluated against the risk of side effects, in particular the combination of mTOR and MAPK inhibitors [71,72].

What are the genetic alterations leading to activation of mTOR signaling and cell-autonomous resistance? Cancer cell lines exposed to increasing doses of gefitinib exhibit *MET* (receptor tyrosine kinase) gene amplification, which in turn leads to PI3K–mTOR pathway activation and gefinitib resistance [73]. Although not equivalent to activating a compensatory pathway, mutation of the FRB domain in mTOR in a human thyroid carcinoma conferred resistance to the allosteric mTOR inhibitor everolimus (rapamycin), possibly accounting for the patients' relapse [74]. Although little is known about the mTOR activating mutations that confer cell-autonomous resistance, these studies suggest that such alterations occur within *MTOR* or in a gene encoding an mTOR regulator.

mTOR Signaling in Non-Cell-Autonomous Resistance

mTOR signaling can also confer resistance to targeted drugs in a non-cell-autonomous manner (Figure 3). In this case, activation of mTOR in cells of the tumor microenvironment confers

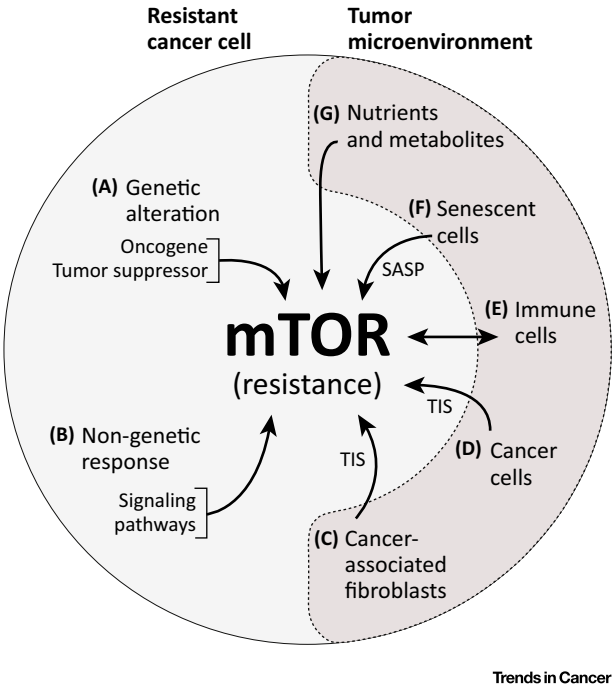

Figure 3. mTOR Confers Resistance in Response to Changes in Cancer Cells or in Stromal Cells (Tumor Microenvironment). Resistance to a targeted therapy can be inherent to the cancer cell or rely on the tumor microenvironment. (A,B) Cancer cell-inherent mechanisms of resistance can be genetically or non-genetically determined. Genetic alterations that activate mTOR to confer resistance may be loss of a tumor-suppressor or acquisition of an oncogene. Non-genetic responses that confer resistance may be dynamic, compensatory signaling changes induced by the drug.

A tumor is a mixture of transformed and non-transformed cells supported by an extracellular matrix, which together form the so-called tumor microenvironment. (C,D) Cells of the tumor microenvironment, in addition to cancer cells, include immune, vasculature, and lymphatic cells as well as cancer-associated fibroblasts (CAFs). Antitumor drugs affect both cancer cells and other cells of the microenvironment. In response to a drug the cells of the tumor microenvironment, such as CAFs or cancer cells that respond to the drug, secrete poorly defined factors referred to as the TIS (therapy-induced secretome). The TIS can act in a paracrine manner to promote resistance. (E) Immune cells play an important role in tumor eradication. mTOR in cancer cells promotes the expression of PD-L1 to suppress tumor-invading immune cells. (F) mTOR in senescent cells regulates the senescence-associated secretory phenotype (SASP). SASP is composed of various cytokines, growth factors, and proteases that modulate the tumor microenvironment. mTORC1 mediates SASP secretion from senescent cells that promotes the resistance and proliferation of nearby cancer cells. (G) Cancer cells operate in different metabolic compartments and secrete metabolites in response to targeted drugs. Nutrients and metabolites in the tumor microenvironment may activate mTORC1 to confer resistance.

resistance on nearby cancer cells. The cells of the microenvironment that confer resistance in a paracrine manner can be stromal cells or cancer cells. Non-autonomous resistance is a drug-induced stress response that is context-dependent and may disappear once the drug (stress) is removed.

Tumors are a heterogeneous population of cells, composed of cancer cells and supporting stromal cells. The therapy-induced secretome (TIS) is a collection of ill-defined factors that are secreted in response to therapy. Stromal TIS can induce extensive changes in the tumor niche to confer drug resistance on nearby cancer cells [75–77]. In this case, the TIS from cancer-associated fibroblasts (CAFs) promotes RTK phosphorylation and thereby mTOR activation in neighboring colorectal or pancreatic cancer cells. In melanoma, the cancer cells respond to the targeted therapy and secrete the TIS component mitogen FOS-related antigen 1 (FRA1). FRA1 activates PI3K–mTOR signaling and promotes resistance in neighboring cancer cells [78]. Finally, mTORC1–4EBP1 signaling controls the TIS in CAFs derived from human pancreatic

tumors [79]. Thus, mTOR plays a dual role in controlling the tumor microenvironment, in other words in sending and receiving the TIS signal to promote cancer resistance to targeted drugs.

Therapy also induces a so-called senescence-associated secretory phenotype (SASP) that can modulate the tumor microenvironment. Therapy may induce cancer cells to secrete SASP factors that are tumorigenic by blunting the effect of the drug on other (non-senescent) cancer cells [80,81]. Two recent studies showed that mTORC1 in senescent cancer cells mediates SASP. Rapamycin selectively abrogates SASP, and thereby improves therapy response in prostate [82] and liver [83] tumor xenografts. This suggests that mTOR can modulate the cancer microenvironment by promoting SASP, and thereby confer therapy resistance in a non-cell-autonomous manner.

Cancer cells present programmed death ligand 1 (PD-L1) to the T cell-borne receptor PD-1. This results in suppression of the T cell, thereby allowing tumor cells to evade killing by the immune system. PD-1 or PD-L1 inhibition, so-called immunotherapy, prevents cancer cells from evading the immune system and is thus an anticancer therapy, particularly effective in the treatment of melanoma. However, PTEN deficient melanomas in which PI3K–mTOR signaling is hyperactive are resistant to immunotherapy. In this context, co-treatment with a PI3K inhibitor improves the efficacy of at least PD-1 inhibition [84]. Furthermore, mTORC1 signaling drives PD-L1 expression in a rapamycin-sensitive manner in mouse models of non-small cell lung carcinoma [85]. Thus, PI3K–mTOR signaling in cancer cells mediates immune evasion and thereby tumor resistance to immunotherapy.

Tumors display complex spatial organization. Cancer cells operate in different metabolic compartments within a tumor and communicate through released metabolites or nutrients. Sonveaux *et al.* [86] showed that cancer cells in hypoxic regions of a tumor perform aerobic glycolysis and consequently excrete lactate. Neighboring cancer cells in normoxic regions of the tumor take up the lactate, via monocarboxylate transporter 1 (MCT1), and utilize it for oxidative respiration. This phenomenon in which tumor cells feed other tumor cells is referred to as metabolic symbiosis. Anti-angiogenic cancer therapy partly disrupts blood vessels, thereby creating hypoxic and normoxic compartments in tumors. Recently, three groups demonstrated that angiogenesis inhibitors induce metabolic symbiosis [87–89]. Importantly, the drug-induced metabolic symbiosis is mTOR-dependent and confers drug resistance [87,88]. Glutamine-activated mTORC1 promotes MCT1 expression and, in turn, lactate uptake in normoxic cells [87,90]. Thus, the normoxic cells utilize lactate as a carbon source, sparing the available glucose for the hypoxic, glycolytic cells that symbiotically feed the normoxic, oxidative cells. The net effect is that cancer cells both near and far from blood vessels survive anti-angiogenic therapy. Rapamycin administration disrupts therapy-induced metabolic symbiosis, leading to tumor regression [87,88]. Furthermore, mTORC1 confers resistance to the glycolysis inhibitor 2-deoxyglucose (2-DG), in a glutaminolysis-dependent manner [91]. In summary, mTORC1 promotes metabolic symbiosis to confer adaptive resistance to angiogenesis inhibitors in a non-cell-autonomous manner.

Concluding Remarks

mTOR signaling is emerging as a major compensatory pathway allowing tumors to escape targeted cancer therapies. mTOR may be a common escape route because it is a central signaling hub functionally related to other oncogenic pathways. Resistance mechanisms can be cell-autonomous or non-cell-autonomous. Non-cell-autonomous resistance is generally adaptive, reversible, and dependent on the tumor microenvironment. Drugs modify the tumor microenvironment, not only the targeted cancer cells. In particular, they stimulate stromal cells and cancer cells to secrete factors that can confer drug resistance to neighboring tumor cells. Importantly, mTOR can mediate both the secretion of such factors and the response to the

Outstanding Questions

What are the cell-autonomous genetic alterations that lead to the activation of mTOR in response to a targeted drug, thereby conferring resistance?

What are the mTOR-dependent non-cell-autonomous changes that could be clinically exploited to limit resistance in cancer cells?

Resistance to a targeted drug is also determined by the spatial and cellular complexity of the tumor. What approaches should be taken to decipher tumor complexity, especially in human tumors?

How can one monitor mTOR activity longitudinally in human patients undergoing targeted therapy?

What regimen should be used for an mTOR inhibitor as a co-therapy?

Box 1. The Use of Phosphoproteomics To Study Mechanisms of Resistance to Targeted Cancer Drugs

Genomic analysis of various human cancers has identified key driver and resistance-conferring mutations, often affecting kinases in signaling pathways [92]. Whereas genomic analysis has been very effective, it only indirectly examines oncogenic signaling pathways. To elucidate signaling changes in response to targeted drugs, phosphorylation cascades can be monitored directly. Mass spectrometry (MS)-based proteomics, in particular phosphoproteomics, is a powerful tool to monitor directly the effect of targeted drugs on oncogenic signaling pathways [93]. However, caution should be taken when performing phosphoproteomics to avoid complications due to rapid dephosphorylation [94]. Phosphoproteins belonging to the MAPK and mTOR signaling pathways are particularly sensitive to ischemia [95] or hypoglycemia. Dynamic signaling cascades are best monitored by analyzing immediately snap-frozen needle biopsies in which tumor conditions are preserved [96].

The usefulness of phosphoproteomics in elucidating resistance mechanisms is underscored by a recent study by Wei *et al.* [97]. Mice transplanted with patient-derived GBM cells were treated with mTOR inhibitors. Cancer cells that developed resistance were then subjected to genomic and phosphoproteomic analyses. Phosphoproteomics revealed marked deregulation of mTOR-related signaling pathways in resistant tumors, suggesting a rewiring of protein signaling networks. However, in-depth genomic analysis did not identify significant genetic changes in resistant tumors versus non-resistant tumors. In another recent study, Dazert *et al.* [50] performed phosphoproteomics on serial biopsies from a sorafenib-treated hepatocellular carcinoma (HCC) patient, taken before and during treatment, to identify mechanisms of resistance to sorafenib. Sorafenib acts by inhibiting RAF (B and C), vascular endothelial growth factor receptor (VEGFR), and platelet-derived growth factor receptor (PDGFR) [98]. Sorafenib is the only approved targeted drug for HCC, with median enhanced survival of <3 months [99]. Dazert and colleagues demonstrated that sorafenib was effective in inhibiting its target in the tumor, based on reduced RSK phosphorylation downstream of BRAF–MEK–ERK signaling. However, phosphorylation of the putative MAPK target Filamin A S2152 and the mTORC1 target S6-S240 was increased in the sorafenib-treated tumor, indicating that a compensatory pathway(s) may have been active in the sorafenib-resistant tumor. Phosphoproteomic analysis of a cohort of patients will provide a more complete picture of the mechanisms of sorafenib resistance.

factors in a recipient cancer cell. The seemingly central role of mTOR in conferring therapy resistance suggests that effective therapy may require combination of an inhibitor of the primary tumor driver and an mTOR inhibitor as co-therapy. To prevent resistance to the co-therapy, intermittent administration should be considered.

Mechanisms of adaptive or intrinsic resistance to targeted drugs are poorly characterized (see Outstanding Questions). The identities of the compensatory signaling pathways and the functional interconnections that underlie resistance are largely unknown. Whereas genomic analysis has been very effective in identifying oncogenic pathways, elucidating the dynamic pathways that confer resistance may require a combination of genomic and phosphoproteomic analyses (Box 1). In particular, tumor biopsies obtained before and during treatment in a longitudinal study should be assessed by mass spectrometry to determine drug-related changes in dynamic phosphorylation cascades. Tumor heterogeneity is a major limitation, especially when human biopsy specimens are limited. Efforts from computational biologists will be important in resolving this complexity.

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