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Extra Views

Reversing Drug Resistance In Vivo

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ABBREVIATIONS

eIF4E	eukaryotic translation initiation factor 4E
mTOR	mammalian target of rapamycin
PI3K	phosphatidylinositol 3-kinase
PTEN	phospatase and tensin homolog
TSC1/2	tuberous sclerosis 1/2 gene

ABSTRACT

Apoptotic defects occur in oncogenesis and contribute to drug resistance. We have shown that Bcl-2, Akt, and the translational regulator eIF4E cooperate with Myc during lymphomagenesis and are potent inducers of drug resistance. Interestingly, lymphomas expressing Akt, but not those expressing Bcl-2 are sensitized to chemotherapy-induced apoptosis by the mTOR inhibitor rapamycin, an effect that is countered by eIF4E. These results provide in vivo validation for a strategy to reverse drug resistance in human cancers and highlight the potential role of translational deregulation in oncogenesis and resistance. They also illustrate the importance of tailoring cancer therapy based on tumor genotype.

Apoptotic defects are a common event in oncogenesis, as disruption of apoptotic programs is required to counteract the pro-apoptotic effects of oncogene signaling and other stresses encountered during tumorigenesis.[1] Since chemotherapeutic drugs also engage these apoptotic pathways, their disruption frequently causes drug resistance.[2] In many cancers, apoptosis is disabled through mutations that ultimately produce hyperactivation of survival signaling molecules such as Bcl-2 or Akt. As a consequence, there are now intense efforts to understand and short-circuit the effects of deregulated survival signaling in cancer.

Our laboratory examines how oncogene and tumor suppressor networks that control cell survival are disrupted during the course of tumor evolution and how mutations in these networks impact tumor cell responses to conventional and targeted therapeutics in vivo. To this end, we have used the Eμ-*myc* mouse model of B-cell lymphomagenesis—not to study lymphomagenesis and lymphoma therapy per se—but to exploit an extremely powerful system for studying genetic interactions relevant to tumorigenesis and treatment responses in vivo. To facilitate these efforts, we developed stem cell manipulation/transplantation protocols to rapidly produce lymphomas with compound genotypes, and employed methods to study treatment responses using real endpoints such as remission and survival in a manner that parallels human clinical trials. Using this system, we have previously shown that disruption of apoptosis, for example through loss of *p53* or overexpression of Bcl-2, dramatically accelerates *myc*-induced lymphomagenesis and promotes drug resistance. Moreover, despite the fact that lymphomas are initiated with the same oncogene and treated in the same way, we note an enormous heterogeneity in response, which is dependent on other genetic alterations in the tumor.

We recently examined the consequences of deregulated Akt signaling on lymphomagenesis and treatment responses in the Eμ-*myc* model. The PI3K/Akt pathway is activated in many tumors through loss of the tumor suppressors PTEN, TSC1/2 or via amplification of Akt or constitutive activation of *ras* (reviewed in ref. 3). Normally the PI3K/Akt pathway transmits growth and survival signals from surface receptors to affect cellular physiology in multiple ways. However, when we directly compared overexpression of Akt to the strictly anti-apoptotic *bcl-2*, we found that both oncogenes had an identical effect on *myc*-induced lymphomagenesis. Both caused the rapid onset of aggressive, multidrug resistant lymphomas of a primordial B cell type. Therefore, despite the myriad of effects Akt has on cellular physiology, its antiapoptotic function appears critical for oncogenesis and drug resistance.

In principle, reversing apoptotic defects through pharmacological or genetic approaches should sensitize drug resistant tumors to cytotoxic therapy. Akt has been proposed to mediate cell survival by phosphorylating proteins directly involved in apoptosis (Caspase 9, Bad, Mdm-2, reviewed in ref. 3), but it also signals transcriptional and translational changes that may indirectly promote survival. However, the relevant contributions of each effector process to oncogenesis and drug resistance, and hence the best molecular targets, remain unclear. Pharmacologically, the choices are currently limited, since Akt inhibitors

are not yet available and drugs targeting the PI3K are toxic. However, one drug—rapamycin —has been widely used as an immunosuppressant in the clinic and is a potent inhibitor of the Akt effector mTOR. Although not typically associated with the Akt-mediated survival signal, mTOR inhibitors are being developed as anti-cancer drugs and have shown some activity towards tumors with defects in the PI3Kinase pathway both in vitro and in vivo.[4-7] Therefore, we tested whether rapamycin could reverse the Akt mediated apoptotic block in our system.

While rapamycin alone had little effect on lymphomas of any genotype, it markedly sensitized Akt overexpressing tumors to cytotoxic chemotherapy.[8] In combination with chemotherapy, rapamycin induced massive apoptosis and lasting remissions without causing increased toxicity. Therefore, targeting a survival pathway can indeed reverse drug resistance in tumors.

Do our studies predict that rapamycin combinations should be universally beneficial? Clearly, they reveal that rapamycin combined with conventional chemotherapy can be synergistic in eliminating tumor cells with mutational activation of the Akt pathway and, indeed, others have observed similar results using targeted drugs.[9] Our results also raise the possibility that rapamycin may enhance drug sensitivity when Akt is activated by growth or survival factors that would otherwise create local sanctuaries of drug resistant cells. Unfortunately, our experiments do not support a broad use of rapamycin to combat drug resistance, since its beneficial effects did not extend to tumors with other apoptotic defects. Furthermore, in chemosensitive control tumors lacking the ARF tumor suppressor but retaining p53, the combination of rapamycin and doxorubicin was antagonistic. This effect occurred only if rapamycin preceded doxorubicin treatment (unpublished observations), and may be the consequence of a rapamycin induced cell cycle arrest.[10] Clearly, these findings imply that rapamycin/chemotherapy combinations are not a 'one-size-fits-all' tumor therapy, but require careful, molecular identification of patients who would benefit from a therapy targeting the PI3K/Akt pathway.

The fact that rapamycin—a specific inhibitor of the translational regulator mTOR—potently reverses the Akt survival signal suggests that control of translation may be important for Akt-mediated cell survival. Consistent with this idea, eIF4E—a translation factor downstream of mTOR—can co-operate in the transformation of primary cells and block apoptosis induced by *myc* or *ras* in vitro.[11-13] Also, in a glioblastoma model, expression of Akt induced translational effects, evident as the rapamycin-sensitive recruitment of certain capped mRNAs into the polyribosome fraction.[14] However, it is also possible that Akt driven lymphomas become "addicted" to the mTOR signal and undergo catastrophic death in its absence. Such a possibility implies that the increased chemosensitivity of the Akt tumors in the presence of rapamycin does not reflect actual reversal of an apoptotic defect, but rather a hypersensitivity to the loss of mTOR signaling.

To further investigate the role of translational deregulation in oncogenesis, we tested the translation initiation factor eIF4E (reviewed in ref. 15) directly in our system. Indeed, we found that eIF4E co-operates with *myc* in a manner that is comparable to Akt, leading to the rapid development of lymphomas. Furthermore, expression of eIF4E was sufficient to confer rapamycin resistance to Akt tumors. This effect was mediated by eIF4E's ability to block apoptosis and compensate for loss of *p53* in tumorigenesis.[8] Consistent with our results, ubiquitous expression of eIF4E in transgenic mice promotes tumorigenesis in the lung, liver and hematopoietic compartment, and co-operates with *myc* in lymphomagenesis.[16] Clearly, the translation factor eIF4E is oncogenic in vivo and sufficient to replace either Akt or *p53* loss in *myc*-driven tumors, and implies that translational regulation can mediate—or at least compensate for—the Akt survival signal.

Do these results mean that only the eIF4E-mediated translational effects are important for Akt's function? Not necessarily. Indeed, it will be interesting to see whether eIF4E can co-operate in oncogenesis with, e.g., S6-kinase, inactivation of caspases or Bad, or how eIF4E's effects on translation intersect with the metabolic alterations that distinguish the survival activities of Akt and Bcl-x_L in FL5-12 cells.[17] Conversely, non-Akt mediated signals may also intersect at the level of eIF4E regulation. For example, the PIM-2 kinase produces a rapamycin/mTOR independent signal that can activate eIF4E.[18] Presumably, the relevant contribution of different survival pathways and their effectors may depend on the cellular context. Yet, together with recent evidence implying defects in mRNA processing in dyskeratosis[19] (reviewed in ref. 20) and amplification of eIF4E in several cancers,[21-23] the surprising ability of eIF4E to impact lymphomagenesis in our model hints at a broader role for translational deregulation in cancer.

OUTLOOK

Although our study provides a compelling example of how reversing apoptotic defects can also reverse drug resistance, several important questions remain. To what extent do Eμ-*myc* lymphomas reflect the behavior of human tumors? If clinical studies recapitulate our results, this will validate genetically controlled tumor models, for uncovering drug resistance mechanisms and as preclinical models for testing new therapeutic approaches. Which patients will benefit from an mTOR-based therapy and how can they be identified? In our study, lymphomas could have virtually identical pathology yet respond very differently to rapamycin—presumably molecular markers that reflect tumor genotype will be required. Are there better drug targets in the Akt pathway? Based on our model, mTOR is a good target, but others may be better. For example direct inhibition of Akt would target a broader range of effectors while, conversely, inhibiting translational initiation downstream of mTOR may provide less opportunities for resistance. However, it is also possible that interfering with upstream and downstream targets may increase toxicity. Finally, can other survival pathways be targeted? Presumably yes, as Bcl-2 antisense oligonucleotides have shown promise in preclinical and clinical studies (reviewed in ref. 24). Clearly, targeted therapeutics hold great promise in cancer therapy and genetically controlled tumor models, like the Eμ-*myc* model, may prove to be particularly valuable in their perclinical development.

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