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Role of mTOR-Chk1 in enhancing DNA-damaging therapy

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Mechanistic target of rapamycin (mTOR) is an atypical Ser/Thr protein kinase that acts as a cellular hub integrating cell growth signals and nutrient availability to promote cell survival.[1] mTOR is the catalytic component of 2 functionally distinct complexes (mTORC1 and mTORC2), and belongs to the family of phosphatidylinositol(3)-like kinases (PIKKs) together with ATM, ATR and DNA-PKcs. The latter 3 kinases have well established roles in the DNA damage response (DDR) – the signaling network activated by DNA damage to promote cell survival by enhancing DNA repair and delaying cell cycle progression in order to maximize repair. However, the participation of mTOR in these pathways has only recently been recognized, aided by the generation and availability of ATP-competitive mTOR kinase inhibitors. A better understanding of the regulation of the DDR is not only vital to understand aspects of tumor development, but is also important to improve DNA damage-based cancer therapies by targeting specific aspects, such as mTOR signaling.

mTOR may act to promote cell survival either by regulating DNA repair and/or cell cycle arrest. Indeed, recent work has demonstrated a role for mTOR kinase in DNA repair through the regulation of FANCD2, a key protein involved in the repair of double-strand DNA breaks.[2,3] Our recent work in Oncotarget[4] also implicates mTOR in control of the cell cycle arrest aspect of the DDR. We found that mTOR is required for efficient establishment of S and G2/M cell cycle arrest after DNA damage, since ablation of mTOR resulted in a reduction in the percentage of cells in S and G2/M. Interestingly, we found that mTORC2 was required for the DNA damage-induced activation of Chk1, a key regulator of cell cycle arrest. We found that mTOR inhibition significantly inhibited all 3 DNA damage-induced phosphorylations of Chk1 (S296, S317 and S345) and reduced the production of Chk1 protein. Since mTOR inhibition consistently ablated DNA damage-induced Chk1 phosphorylation under all conditions tested, whereas inhibition of protein levels was cell type specific, this suggests that there may be distinct mechanisms by which mTOR controls Chk1 and that phosphorylation of Chk1 may be of primacy. Exactly how mTOR might regulate Chk1 activity is an open question. One likely candidate could be via ATR, since its ability to phosphorylate Chk1 at Ser317 and Ser345 leading to autophosphorylation at Ser296 is well established. Alternatively, the effects on Chk1 might involve one of the substrates of mTORC2. Our results support accumulating evidence from studies in yeast implicating TORC2 in survival under conditions of DNA damage and in genome stability.[5]

Since approval of the rapalog, Everolimus, in combination with hormone therapies for breast cancer, the potential of mTOR inhibitors to overcome resistance to current therapies has gained increasing interest. Indeed numerous publications have demonstrated that mTOR inhibition can also enhance radio- or chemotherapy-induced cell death in various cancer types. We also investigated the translational significance of our results and found that an mTOR kinase inhibitor enhanced DNA damage-induced cell death in HEK293 cells and a panel of breast cancer cell lines.[4] While this suggests that mTOR kinase inhibitors may be useful therapeutically to augment DNA damage based therapies, it is likely that these effects will be tumor-type specific since similar augmentation was not found in renal and colon cancers.[6,7] We have previously shown that an mTOR kinase inhibitor prevented p53-dependent cell death in renal carcinoma cells.[6] It is known that mTORC1 contributes to DNA damage-induced p53 activation via multiple mechanisms, therefore it is likely that mTOR ablation leads to p53 suppression and inhibition of p53-dependent apoptosis in these renal cells. However, HEK293 cells have non-functional p53 and the panel of breast cancer cell lines that we tested displayed a variety of either wild-type, lost or mutated p53, but all still exhibited chemosensitivity following mTOR kinase inhibition. This suggests that these latter cells may rely on mTORC2-Chk1 signaling for survival. In conclusion, it is clear that mTOR regulates the DDR response by multiple mechanisms. Whether a given tumor type is made chemosensitive or chemoresistant by mTOR kinase inhibitors will depend on which aspect of mTOR signaling is altered during tumorigenesis. In this vein, it will be important to widen our understanding of mTOR targets following DNA damage in order to gain better insight into whether specific targeting of mTORC2 will be a useful adjuvant to DNA-damaging therapies.

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