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Prevention of BMS-777607-induced polyploidy/senescence by mTOR inhibitor AZD8055 sensitizes breast cancer cells to cytotoxic chemotherapeutics

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ABSTRACT

Targeted inhibition of MET/RON signaling by tyrosine kinase inhibitor BMS-777607 for cancer treatment is currently under clinical trials. We have previously shown that BMS-777607 induces chemoresistance *in vitro* by causing polyploidy, which hampers therapeutic efficacy. Here, we studied polyploidy-associated senescence induced by BMS-777607 in breast cancer cells and its prevention by mTOR inhibitor AZD8055, leading to increased chemosensitivity. In breast cancer T-47D and ZR-75-1 cells, BMS-777607 induced phenotypic changes including enlarged cellular size, flattened morphology, increased DNA content, and activity of senescence-associated β-galactosidase. These changes were accompanied by increased p21/WAF1 expression and decreased Retinoblastoma Ser^{780} phosphorylation, indicating that BMS-777607 induces not only polyploidy but also senescence. The appearance of senescence was associated with polyploidy in which β-galactosidase is exclusively expressed in polyploid cells. Survivin expression was increased in polyploid/senescent

Abbreviations: AXL, Greek word anexelekto; BCR, breakpoint cluster region; BRAF, B-Rapidly Accelerated Fibrosarcoma; DAPI, 4′,6′-diamidino-2-phenylindole; EMT, epithelial to mesenchymal transition; EGFR, epithelial growth factor receptor; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; FLT-3, Fms-like tyrosine kinase 3; IGF-1R, insulin-like growth factor receptor-1; mAb, monoclonal antibody; MER, monocytes, epithelial and reproductive tissue; MET, mesenchymal to epithelial transition; mTOR, mammalian target of rapamycin; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; WAF1, wild-type p53-activated fragment 1; PI3K, Phosphatidylinositide 3-kinase; Rb, retinoblastoma; RON, recepteur d'origine nantais; RTK, receptor tyrosine kinase; SABG, senescence-associated β-galactosidase; siRNA, small interfering RNA; TKI, tyrosine kinase inhibitor; TYRO-3, tyrosine protein kinase receptor-3.

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cells as analyzed by Western blotting. Increased survivin accumulated both in the nucleus and cytoplasm and dissociated with condensed DNA and mitotic spindle at the metaphase. Abnormal accumulation of survivin also rendered polyploid/senescent cells insensitive to cytotoxic activities of YM155, a DNA damaging agent with a suppressive effect on survivin gene transcription. AZD8055, a specific mTOR inhibitor, effectively prevented BMS-777607-induced polyploidy and senescence and restored survivin expression and its nuclear localization to normal levels. Although a synergism was not observed, BMS-777607 plus AZD8055 increased cancer cell sensitivity toward different cytotoxic chemotherapeutics. In conclusion, BMS-777607-induced chemoresistance is associated with cell polyploidy and senescence. Inhibition of mTOR signaling by AZD8055 prevents BMS-777607-induced polyploidy/senescence and increases breast cancer cell chemosensitivity.

1. Introduction

Receptor tyrosine kinases (RTK)[2] MET and RON belong to a unique RTK subfamily implicated in epithelial tumorigenesis and malignancy (Gherardi et al., 2012; Yao et al., 2013a,b). In breast cancer, overexpression of MET and RON is a pathogenic factor that facilitates the tumorigenic progression and provides the prognostic value for patient survival (Beviglia et al., 1997; Lee et al., 2005; Knight et al., 2013; Kretschmann et al., 2010). Moreover, increased MET and RON expression has been validated as a drug target for breast cancer therapy (Blumenschein et al., 2012, Yao et al., 2013). Various TKIs such as foretinib, MGCD265, and MK8033 and therapeutic monoclonal antibodies including onartuzumab and IMC-RON8 (also known as Narnatumab) have been developed (Choueiri et al., 2013; Eder et al., 2010; Belalcazar et al., 2012; Northrup et al., 2013; Merchant et al., 2013; Zou et al., 2013). Currently, these TKIs and therapeutic antibodies are under clinical trials for advanced cancers that harbor aberrant MET and/or RON signaling. Preliminary results from these clinical studies indicate potential for tumor growth control and prolonged patient survival.

The major challenge faced in targeted cancer therapy is the development of acquired resistance to specific TKIs or therapeutic antibodies (Shien et al., 2013; Wheeler et al., 2010). Although clinical trials are still underway, acquired resistance to MET and RON dual TKIs has been reported in preclinical studies (Diamond et al., 2013; McDermott et al., 2010; Cepero et al., 2010; Qi et al., 2011). Currently, three major mechanisms of acquired resistance have been identified in kinase-targeted cancer therapy (Engelman and Jänne, 2008; Garraway and Jänne, 2012). Genetic alterations including point mutation, gene amplification and alternative mRNA splicing are the forms of acquired resistance occurring in target proteins such as MET, EGFR, BCR-ABL, and p16BRAF (Engelman and Jänne, 2008; Garraway and Jänne, 2012). In MET, a point mutation in the kinase activation loop (Y^{1230}) destabilizes the autoinhibitory conformation of the protein leading to the hyper-activation status (Qi et al., 2011). Activation of an alternative signaling pathway(s) also increases survival of cancer cells under targeted conditions (Engelman and Jänne, 2008; Garraway and Jänne, 2012). In lung cancers, acquired resistance caused by gefitinib-induced inhibition of EGFR is featured by activation of the MET pathway with increased PI3K-AKT signaling (Engelman et al., 2007). In targeting IGF1R for the treatment of childhood sarcoma, increased RON expression and signaling have emerged as a compensatory mechanism for the survival of tumor cells (Potratz et al., 2010). In addition, activation of the EGFR pathway or induction of KRAS gene amplification has been found to mediate acquired resistance to MET/RON targeted TKIs (Cepero et al., 2010). Another mechanism involved in acquired resistance is the change of cellular phenotype such as EMT (Kim et al., 2013; Ahmed et al., 2010). Cancer cells with EMT are highly resistant to TKI-targeted therapy due to their acquisition of certain stem/progenitor cell characteristics, which are known as hallmark featured for drug resistance (Kim et al., 2013; Ahmed et al., 2010). Thus, understanding the mechanisms underlying acquired resistance by cancer cells is urgently needed.

BMS-777607 is a synthetic TKI selective for the MET superfamily (Schroeder et al., 2009). BMS-777607 primarily targets RON (IC_{50}: 1.8 nM), MET (IC_{50}: 3.9 nM), TYRO-3 (IC_{50}: 4.3), and AXL (1.1 nM) (Schroeder et al., 2009). At relatively high concentrations, BMS-777607 also inhibits other targets such as MER (IC_{50}: 14.0 nM), FLT-3 (IC_{50}: 16 nM), and aurora kinase B (AuKB, IC_{50}: 78 nM) (Schroeder et al., 2009). Thus, BMS-777607 is best viewed as a multi-kinase inhibitor. Preclinical studies have shown that BMS-777607 *in vitro* inhibits MET and RON signaling and suppresses various tumorigenic activities including cell growth and migration (Schroeder et al., 2009; Dai and Siemann, 2010; Sharma et al., 2013). Studies from tumor xenograft models also confirm that BMS-777607 effectively inhibits tumor growth in a dose-dependent manner (Schroeder et al., 2009). However, BMS-777607 treatment also causes cancer cell chemoresistance manifested by the off-target effect (Sharma et al., 2013). We have previously shown that treatment of breast, colon, and pancreatic cancer cells *in vitro* with BMS-777607 induces extensive polyploidy. This effect is caused by inhibition of AuKB, resulting in cell cycle arrest at pro-metaphase and failure to undergo cytokinesis (Sharma et al., 2013). Polyploid cells are long-lived and acquire resistance to cytotoxic chemotherapeutics (Sharma et al., 2013; Davis et al., 2008). Thus, BMS-777607-induced phenotypic change owing to its off-target effect opens a pathogenic avenue leading to acquired chemoresistance. In other words, the off-target effect could constitute a mechanism of acquired resistance in targeted cancer therapy.

The present study seeks to find a pharmacological means to prevent BMS-777607-induced chemoresistance and to increase the therapeutic efficacy of BMS-777607 against cancer