Senescence as a Mechanism of Resistance to Aurora Kinase Inhibition with ENMD-2076 in p53 mutated Triple-Negative Breast Cancer (TNBC) Models

Anastasia A. Ionkina1, S. Gail Eckhardt1, Todd M. Pitts1, Jiye Kim1, Aik Choon Tan1, Carol Sartorius2, Peter Kabos1, John J. Tenter1, Jennifer R. Diamond1

1Division of Medical Oncology, 2Department of Pathology, University of Colorado Denver Anschutz Medical Campus, Aurora CO

ABSTRACT

In triple-negative breast cancer (TNBC), the lack of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression results in a heterogeneous tumor population making the disease difficult to treat. ENMD-2076 is a potent Aurora A/B inhibitor that has shown promise in preclinical studies for TNBC, however limited data exist on the role of p53 mutations in the development of resistance. Microarray and RNA sequencing were performed to identify drug sensitive and resistant TNBC models. ENMD-2076 was found to induce cell cycle arrest and senescence. The induction of senescence in the resistant models could be associated with loss of functional p53.

INTRODUCTION

Triple-negative breast cancer (TNBC) is an aggressive type of breast cancer with a high rate of metastasis and mortality. The lack of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression results in a heterogeneous tumor population making the disease difficult to treat. ENMD-2076 is a potent Aurora A/B inhibitor that has shown promise in preclinical studies for TNBC. Although the drug has demonstrated anti-tumor activity, limited data exist on the role of p53 mutations in the development of resistance. Microarray and RNA sequencing were performed to identify drug sensitive and resistant TNBC models. ENMD-2076 was found to induce cell cycle arrest and senescence. The induction of senescence in the resistant models could be associated with loss of functional p53.

MATERIALS AND METHODS

Three TNBC preclinical models harboring different p53 mutations were used for ENMD-2076 treatment. Sensitivity to ENMD-2076 was assessed using tissue microarrays as well as xenografts (PDTX). Freshly harvested PDTX samples were placed into individual cryomolds (Sakura Finetek, Torrance, CA) and embedded in OCT Compound (Sakura). RNA from the PDTX models was extracted using the NucleoSpin® RNA Kit (Macherey-Nagel, Bethlehem, PA). mRNA sequencing was performed using the NextSeq 500 (Illumina). RT-qPCR was carried out to measure gene expression and western analysis was performed to determine the status of p53. Immunofluorescence staining was performed on serial sections of PDTX to demonstrate p53, p16, phospho Aurora A, phospho AKT, and phospho JNK expression. Immunohistochemistry was performed on a representative of the p53 mutated TNBC models.

RESULTS

We have previously shown ENMD-2076 to induce cell cycle arrest and senescence, which can be associated with loss of functional p53. Intrinsic and acquired resistance to ENMD-2076 in TNBC PDTX models is associated with loss of p73 expression and an increase in markers associated with senescence. The reduced sensitivity of p53 mutated TNBC models compared to wild type is consistent with the role of p53 in mediating response to Aurora kinase inhibition. In the absence of functional p53, the intrinsically resistant model CU_TNBC_004. These findings point to genes associated with the development of senescence.

CONCLUSIONS

• ENMD-2076 exhibits differing degrees of antiproliferative activity towards TNBC PDTX models.
• We observed an increase in apoptosis in response to ENMD-2076 treatment at day 30 in CU_TNBC_002 and CU_TNBC_005 PDTX models, whereas CU_TNBC_004 PDTX model was resistant to ENMD-2076 treatment (p<0.05). These findings point to genes associated with the development of senescence.
• This study supports the role of p53 in mediating response to Aurora kinase inhibition in TNBC. Understanding the role of mutant p53 in mediating response to Aurora kinase inhibition in TNBC may lead to new therapeutic opportunities.

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