Aryl hydrocarbon receptor agonist ZSTK3744 overcomes chemotherapy resistance in TNBC

#5643

Takahiro Ohashi[1], Kayoko Kawai-Asami[2], Tsubasa Nagasaki[3], Kotomi Akatsuka[1], Yuko Nagata[2], Sayuri Terada[1], Shinsuke Hiramoto[1], Eika Higashi[3], Ryuichiro Ohshita[3], Keiko Fukushima[1]

[1]Biosciences, [2]Pharmacology, [3]Organic Synthesis, Department of Drug Discovery, R&D Center, Zenyaku Kogyo Co., Ltd., Tokyo, Japan.

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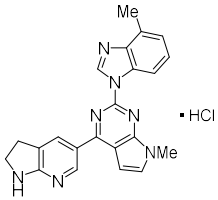
Introduction

Systemic chemotherapy remains the primary treatment option for TNBC. However, its benefits are limited, and some patients acquire resistance, further complicating treatment. New therapeutic approaches are urgently needed to overcome chemotherapy resistance in TNBC. ZSTK3744, an aryl hydrocarbon receptor (AhR) agonist, has been developed as a novel therapeutic candidate for TNBC (Poster: 5631). In this study, we evaluated the anti-tumor effects of ZSTK3744 on chemo-resistant TNBC cells and compared its efficacy and toxicity with other AhR agonists undergoing clinical trials.



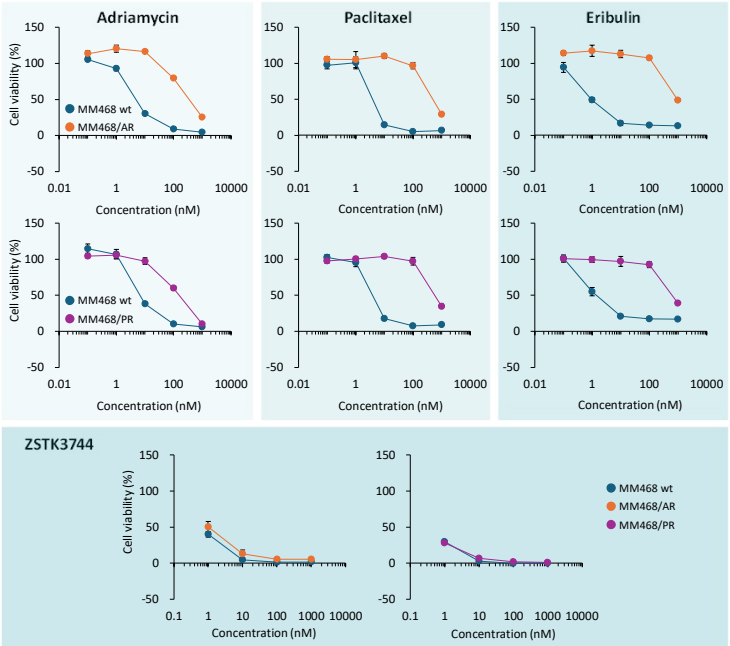
ZSTK3744

Results

1. Establishment of chemo-resistant cells

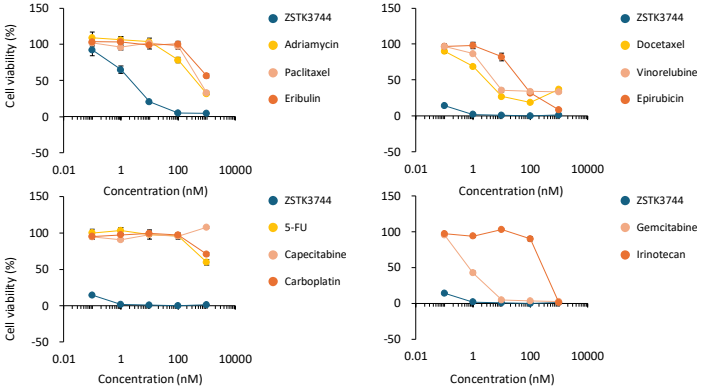
MDA-MD-468 (TNBC cell line, MM468) cells were exposed to adriamycin or paclitaxel at concentrations of 10 to 140 nM and 2 to 320 nM, respectively. Surviving cells were designated as adriamycin-resistant (AR) and paclitaxel-resistant (PR) MM468 cells, respectively.

2. Assessment of anti-tumor effects on resistant cells



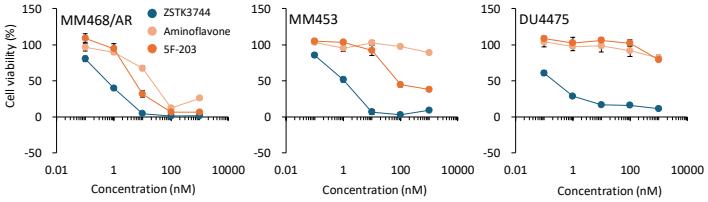
Parental MM468, MM468/AR, and MM468/PR cells, were treated with adriamycin, paclitaxel, eribulin or ZSTK3744 at the indicated concentrations for 72 h. Cell viability was assessed using the Cell Counting Kit-8 assay. The results are presented as the mean ± SD of quadruplicate.

3. Anti-tumor effects of ZSTK3744 on MM468/AR cells



MM468/AR cells were treated with standard chemotherapeutic agents or ZSTK3744 at the indicated concentrations for 72 h. The results are presented as the mean ± SD of quadruplicate.

4. Comparison of the effects of different AhR agonists



MM468/AR, MM453 and DU4475 (TNBC cell lines) cells were treated with aminoflavone, 5F-203, or ZSTK3744 at the indicated concentrations for 72 h. the results are presented as the mean ± SD of quadruplicate.

5. RNA-seq analysis of chemo-resistant cells

MM468/AR

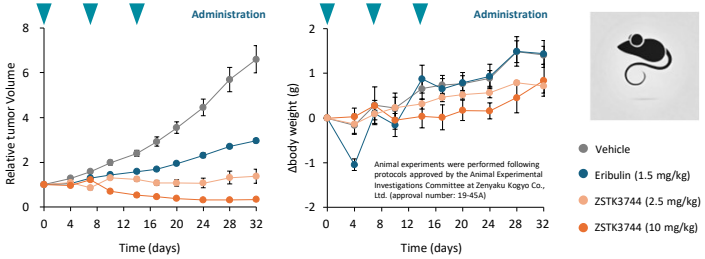
Upregulation			Downregulation		
Gene symbol	Fold changes	P-value	Gene symbol	Fold changes	P-value
ABCB1	572.5	6.E-10	*SGCD*	-110.4	3.E-05
CSF3R	137.9	5.E-06	*STRA6*	-90.0	6.E-06
GPR87	127.2	3.E-05	*GABBR2*	-75.2	3.E-05
CPS1	113.6	5.E-06	*SLC28A3*	-59.8	3.E-05
NNMT	91.0	5.E-06	*SHISA2*	-49.3	8.E-04
COL12A1	81.4	5.E-06	*PLEKHS1*	-45.0	1.E-04
PDZD4	73.6	7.E-05	*C2orf72*	-43.5	9.E-05
SERPINB2	66.9	7.E-05	*PTPRM*	-42.4	1.E-03
ZDHHC2	63.6	5.E-05	*CP*	-40.5	4.E-02
CPQ	62.6	8.E-05	*MUC16*	-35.2	3.E-04
PCDHB16	59.0	6.E-03	*HSD17B2*	-33.5	4.E-04
HTR7	55.4	2.E-04	*ZBED2*	-32.7	4.E-03
COL26A1	54.1	5.E-04	*DDC*	-32.5	2.E-03
ZNF765-ZNF761	50.5	3.E-04	*ANO1*	-31.8	3.E-04
UGT1A6	49.7	5.E-05	*NCAM1*	-30.8	5.E-04
CLMP	49.4	8.E-05	*PDZK1*	-30.3	6.E-04
MAT1A	47.4	3.E-04	*CAMK4*	-29.3	9.E-04
GULP1	47.2	8.E-04	*GPR12*	-29.3	3.E-03
PCDHA1	46.7	2.E-03	*IRS4*	-29.0	3.E-03
AOX1	43.5	9.E-05	*NEFH*	-27.9	6.E-04
FABP6	43.2	4.E-04	*DIPK2A*	-27.8	9.E-04
MAGED1	39.9	2.E-04	*NEURL3*	-26.8	6.E-04
MSX1	37.0	2.E-03	*COL8A1*	-26.4	4.E-03
CALB2	35.4	2.E-04	*AJM1*	-25.7	1.E-03
PCDHB8	34.7	5.E-04	*COMMD3-BMI1*	-23.8	2.E-03
TP63	31.3	4.E-04	*EDN2*	-22.9	2.E-03
PLA2G4F	30.5	1.E-03	*CHST9*	-22.0	4.E-03
GPM6B	29.6	1.E-02	*SUN3*	-21.3	2.E-03
IL1R2	28.9	9.E-04	*KLHL30*	-20.8	1.E-02
C4orf19	28.1	6.E-04	*GALNT14*	-20.8	6.E-03

MM468/PR

Upregulation			Downregulation		
Gene symbol	Fold changes	P-value	Gene symbol	Fold changes	P-value
ABCB1	534.5	1.E-09	*MYEOV*	-204.4	1.E-07
TCEAL8	199.9	2.E-06	*ELAPOR1*	-182.8	1.E-07
GULP1	179.3	1.E-06	*TVP23C-CDRT4*	-181.7	4.E-05
SLC2A3	160.0	2.E-07	*SLC34A2*	-178.7	1.E-07
RORA	145.8	2.E-06	*SREK1*	-130.5	6.E-07
COL27A1	142.7	1.E-06	*CCL22*	-117.4	1.E-06
DIO2	137.8	2.E-07	*PLEKHB1*	-109.3	4.E-04
TSPEAR	127.1	8.E-07	*ARL11*	-107.8	2.E-05
ZNF765-ZNF761	107.5	4.E-06	*PCDHB*	-102.0	2.E-06
ZDHHC2	105.8	2.E-06	*STRA6*	-94.0	2.E-06
COL12A1	85.2	1.E-06	*NCAM2P*	-86.4	1.E-05
FAT4	73.0	9.E-06	*ATP13A5*	-76.0	4.E-06
CPQ	70.9	3.E-05	*CD14*	-75.4	6.E-06
NRCAM	70.6	6.E-05	*ATP6V0D2*	-75.1	3.E-06
PDZD4	66.4	4.E-05	*CLDN10*	-74.4	3.E-06
SERPINI1	65.6	2.E-05	*ALDH2*	-72.1	5.E-06
GPR63	63.1	3.E-05	*SLC6A14*	-70.6	4.E-06
ZNF275	62.7	3.E-04	*TMEM92*	-69.0	2.E-04
KIF26A	61.0	1.E-04	*ATP6V1B1*	-67.1	4.E-06
IGSF10	59.7	7.E-05	*IQGAP2*	-62.7	3.E-04
JHY	58.9	4.E-04	*MAP7D2*	-60.3	9.E-05
CALB2	58.6	8.E-06	*ARHGEF6*	-59.1	7.E-06
GALNT18	58.0	1.E-04	*CYP4X1*	-58.2	8.E-06
KIF1A	53.8	2.E-05	*LRRC55*	-56.4	4.E-04
CFI	50.8	1.E-04	*MSMB*	-55.0	5.E-04
SLC12A7	48.0	1.E-04	*PRODH*	-53.9	2.E-05
MYH3	44.6	3.E-04	*CAPN13*	-49.8	3.E-05
PCDH18	43.0	5.E-05	*GPR55*	-48.5	2.E-04
H3C4	42.2	6.E-04	*PLEKHS1*	-47.0	3.E-05
ADAMTS5	38.4	6.E-05	*ATP6V0A4*	-45.7	2.E-05

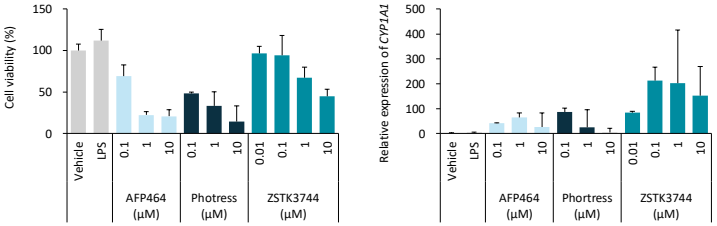
The top 30 significantly upregulated and downregulated differentially expressed genes in MM468/AR and MM468/PR cells compared to wild-type cells.

6. Evaluation of anti-tumor efficacy in xenograft model



NOD-SCID mice were subcutaneously injected with MM468/AR cells. Administration of these drugs was initiated when the tumor volume reached 100–300 mm^3. ZSTK3744 was administered intravenously at doses of 2.5 or 10 mg/kg, whereas eribulin was administered intravenously at a dose of 1.5 mg/kg on days 0, 7, and 14. Tumor volumes and body weight were measured on days 0, 4, 7, 10, 14, 17, 20, 24, 28, and 32 (mean ± SD, n = 5).

7. Evaluation of pulmonary toxicity in PCLS



Human precision-cut lung slices (PCLS) were continuously treated with ZSTK3744 (0.01, 0.1, 1 and 10 μM), AFP464 (0.1, 1 and 10 μM), or Phortress (0.1, 1 and 10 μM) for 7 days. Cell viability was measured using the Cell Counting Kit-8 assay (mean ± SD of triplicate experiments). The mRNA levels of CYP1A1 after treatment with ZSTK3744, AFP464, or Phortress were quantified using RT-qPCR. Data from PCLS experiments were generated at the IIVS.

Conclusion

- ✓ ZSTK3744 exhibited superior and broader anti-tumor efficacy than chemotherapeutics and other AhR agonists in chemo-resistant TNBC cells.
- ✓ ZSTK3744 demonstrated more potent anti-tumor effects than eribulin in xenograft model transplanted with adriamycin-resistant MDA-MB-468 cells.
- ✓ ZSTK3744 demonstrated lower pulmonary toxicity than other AhR agonists in PCLS.

ZSTK3744 is a promising therapeutic candidate for patients with chemo-resistant TNBC.

Reference

Paper : Submitted
Patent: Patent Application No. JP2024-075404

COI

The authors are employed by Zenyaku Kogyo Co., Ltd.