ZSTK3744, a novel AhR agonist, demonstrates antitumor efficacy in TNBC cell lines *in vitro* and *in vivo*

#5631

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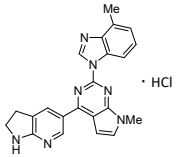
Introduction

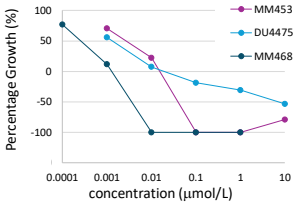
Breast cancer is the most commonly diagnosed cancer among women worldwide. Triple-negative breast cancer (TNBC) is the most aggressive form of breast cancer and has limited treatment options. The absence of key molecular targets in TNBC complicates treatment with conventional therapies, limiting effective therapeutic options. Herein, we report a novel small molecule, ZSTK3744, which exhibits potent inhibitory effects on the proliferation of TNBC-derived cell lines both *in vitro* and *in vivo*. (Related Presentation: #5643)

Results

1. Anti-tumor effects of ZSTK3744 on TNBC cell lines



ZSTK3744



MDA-MB-453 (MM453), MDA-MB-468 (MM468), and DU4475 (TNBC cell lines) cells were treated with ZSTK3744 at the indicated concentrations for 72 h. ZSTK3744 inhibited cell proliferation and induced cell death (percentage growth < 0%) in TNBC.

2. Anti-tumor effects on breast and ovarian cancer cell lines

Cancer		Cell line	GI_{50}(nM)
breast cancer		MCF-7	20
		T47D	0.22
		ZR-75-1	2.7
		SK-BR-3	6.7
		CAMA-1	8.6
		BT-474	3.8
	TNBC	MM468	0.69
		MM453	2.7
		DU4475	1.3
		CAL-51	2.2
		BT-20	12
		MM231	9.0
		MM436	360
		HCC38	1.5
		Hs578T	>1,000
		HCC1937	>1,000

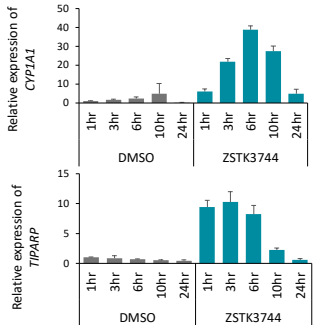
Cancer	Cell line	GI_{50}(nM)
ovarian cancer	RMG-1	1.3
	RMUG-S	45
	OV-90	8.3
	OMC-3	4.4
	OVCAR-3	3.9
	Caov-3	7.3
	A2780	2.4
	ES-2	>1,000
	SK-OV-3	>1,000
	MCAS	>1,000

Cells were treated with varying concentrations of ZSTK3744 for 72 h, and GI_{50} was calculated.

GI_{50} : 50% growth inhibitory concentration

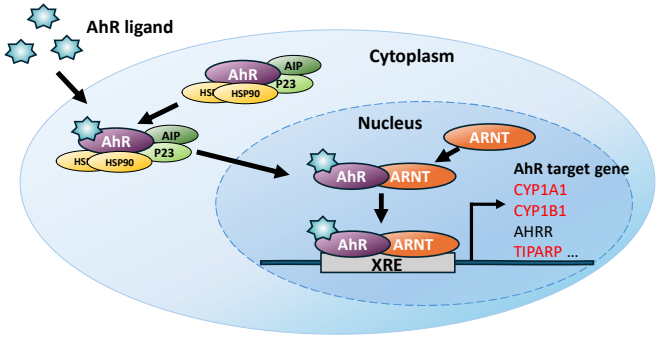
3. DNA microarray analysis in ZSTK3744 treated cells

Gene Symbol	Fold change
CYP1A1	223.4
ATF3	19.0
HBEGF	18.6
CYP1B1	15.3
STC2	13.0
DLX1	12.6
TIPARP	10.4
ID2	10.2
SNAI1	9.4
PMAIP1	8.7
RND1	8.6
SHISA2	8.5
TIPARP	7.6
CHAC1	7.6
RHOB	7.3
MAFF	7.2
SGK1	7.2
DDIT3	6.9
PRDM1	6.7
FOSL1	6.4



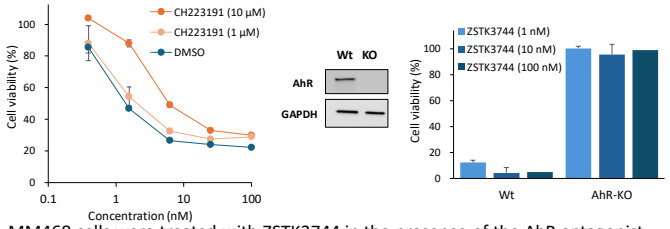
The top 20 genes significantly upregulated in MM468 cells treated with ZSTK3744 (1 μM, 6 h) are shown in the table. Several genes related to the aryl hydrocarbon receptor (AhR) pathway (red) were identified. Among them, sequential changes in CYP1A1 and TIPARP mRNA levels were confirmed by RT-qPCR in MM468 cells treated with 1 μM ZSTK3744.

4. The AhR signal pathway



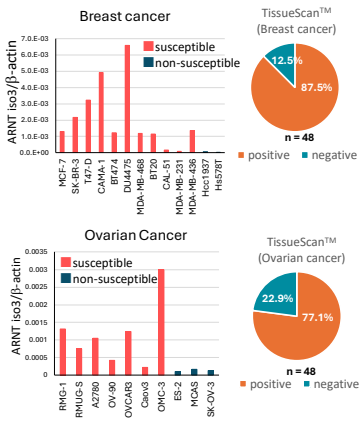
DNA microarray showed that ZSTK3744 activated the AhR pathway. Therefore, we hypothesized that AhR is involved in the mechanism underlying the antitumor activity of ZSTK3744.

5. Investigation of the target molecule of ZSTK3744



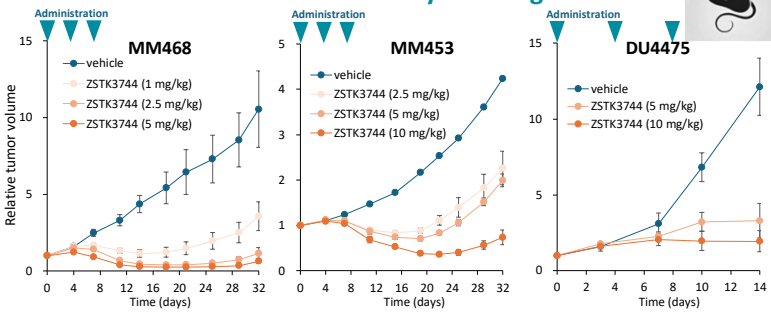
MM468 cells were treated with ZSTK3744 in the presence of the AhR antagonist CH223191. Co-treatment with CH223191 attenuated the anti-tumor effects of ZSTK3744 in a dose-dependent manner (left). Additionally, we used the CRISPR/Cas9 system to generate AhR-knockout (AhR-KO) MM468 cells and confirmed AhR deficiency in these cells by western blot analysis. Cell viability assays showed that AhR-KO MM468 cells had increased resistance to ZSTK3744 compared with parental MM468 cells (right). These results suggest that AhR is essential for the anti-tumor effects of ZSTK3744.

6. Biomarker for ZSTK3744-susceptible cells



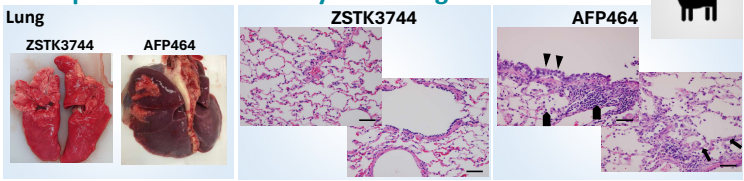
Aryl hydrocarbon receptor nuclear translocator isoform3 (ARNT iso3) has been reported to be a potential biomarker for aminoflavone (AF), an AhR agonist developed as an anticancer drug (patent: US2013/0177904A1). ARNT iso3 mRNA expression levels in ZSTK3744-susceptible/non-susceptible cell lines were measured by RT-PCR. Then, ARNT iso3 mRNA expression was analyzed in cDNA derived from cancer patients. The expression level of the susceptible cell line with the lowest ARNT iso3 expression in each cancer type was used as the reference value, and samples with expression levels above this threshold were considered positive. The results showed that 87.5% of samples from breast cancer patients and 77.1% of samples from ovarian cancer patients were classified as ARNT iso3 positive, suggesting that such patients may be eligible for treatment.

7. Evaluation of anti-tumor efficacy in xenograft model



NOD-SCID mice were subcutaneously injected with MM468, MM453, or DU4475 cells. ZSTK3744 was administered intravenously at doses of 1~10 mg/kg on days 0, 4, and 8. Tumor volumes were measured on days 0~32 (mean ± SE, n = 5, *DU4475: days 0~14, n = 3). Long-term tumor shrinkage was maintained after only three administrations (Relative tumor volume < 1).

8. Comparison of the toxicity of AhR agonists



The toxicity of ZSTK3744 and the AF prodrug AFP464 were evaluated following intravenous administrations in Beagle dogs. ZSTK3744 was administered at escalating doses of 0.384, 0.96, 2.4, 6, and 15 mg/kg (n = 2), whereas AFP464 was administered at a fixed dose of 11.4 mg/kg (n = 1) once weekly. The dog treated with AFP464 exhibited a marked deterioration in condition and was euthanized one day after the second administration. From 4 days after the first administration until euthanasia, the dog displayed persistent panting (rapid, shallow breathing). Gross examination revealed dark reddish discoloration of the lungs, and histological analysis revealed alveolar epithelial regeneration with hypertrophy of type II alveolar epithelial cells (arrows) , bronchial inflammation (five-way arrows), and reactive bronchial epithelial hyperplasia (arrow heads). In contrast, no similar symptoms were observed in the dogs treated with ZSTK3744. Bar_100 μm.

Animal experiments were performed following protocols approved by the Animal Experimental Investigations Committee at Zenyaku Kogyo Co., Ltd. (approval number: 19-45A, 21-1, 21-27)

Conclusion

- ✓ ZSTK3744 demonstrated potent anti-tumor effects in breast and ovarian cancer cell lines and TNBC xenograft models.
- ✓ ZSTK3744 activates AhR, which is essential for its anti-tumor effects.
- ✓ ARNT iso3 could be a biomarker for ZSTK3744.
- ✓ ZSTK3744 is anticipated to have a favorable tolerance profile regarding pulmonary toxicity, a known concern with AhR agonists.

ZSTK3744 is a promising therapeutic candidate for patients with breast and ovarian cancer.

Reference

- Paper submitted
- Patent Application No. JP2024-075404

COI

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