A study on EGFR gene amplification and protein expression in Chinese esophagus cancer patients and anti-tumor activity of an EGFR inhibitor Epitinib in patient derived esophagus cancer models

Yongxin Ren[1], Jianming Zheng[2], Linfang Wang[1], Fang Yin[1], Wei Zhang[1], Jinghong Zhou[1], Xuelei Ge[1], Shiming Fan[1], Renxiang Tang[1], Junen Sun[1], Weiguo Qing[1], Weiguo Su[1]

[1] Hutchison MediPharma Ltd. Building 4, 720 Cai Lun Road, Z.J.Hi-Tech Park, Shanghai, China, 201203; [2] Co-first author, First Affiliated Hospital, Second Military Medical University, 200433

Abstract # 1730

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Introduction

- Esophagus cancer is the fifth most common malignancy and the fourth leading cause of cancer mortality in China. According to the Chinese cancer registry annual report in 2012 [1], esophagus cancer accounts for nearly 1 in 10 of all cancer deaths. Despite the fact that much progress has been made in diagnosis and systemic chemotherapy regimens, the overall prognosis of esophagus cancer continues to be disappointing. The 5-year survival rate, all stages included, is around 15~25% [2]. There remains a significant unmet medical need for esophagus cancer treatment.
- EGFR expression was reported in 30~90% esophagus cancers and overexpression of EGFR was found to be associated with poor survival [3]. Unlike colon cancer, K-ras mutation was less frequently found in esophagus cancer (0~16%) [4], suggesting EGFR pathway blockade might bring therapeutic benefit to those patients with EGFR activation.
- In this study, 43 surgical esophagus tumor samples were collected from Chinese patients, from which, nine patient derived xenograft (PDX) models were developed. Anti-tumor effect of a novel and highly potent EGFR inhibitor Epitinib, currently being evaluated in phase I clinical trials in China, was evaluated in 6 PDX models.

Materials and methods

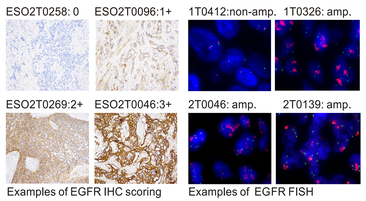
- **Tumor tissues:** Esophagus tumor samples from 43 treatment-naive patients were collected during surgical resection from Shanghai Biobank Network of Common Human Tumor Tissue (supported by SMSTC, Grant No. 12DZ2295100), including 34 frozen tumors and 9 fresh specimen. Freshly harvested specimen were separated into three parts: 1. Implanted into animals; 2. Snap frozen in liquid nitrogen for DNA extraction and sequencing; 3. Prepared FPEE sections for pathological analysis.
- **EGFR IHC staining and scoring:** IHC staining was performed with EGFR PharmDx (DAKO) and the whole section was carefully examined. The staining intensity was scored using a four-tier system. The percentage of tumor cells with positive staining was reviewed and H score was calculated. The categorical score was determined by the intensity score of the largest percentage of tumor cells. Categorical score ⩾2 was regarded as EGFR high expression.
- **EGFR gene amplification: determined by real time PCR and FISH.**
  - **Real time PCR:** PCR was carried out in a 20 L volume including genomic DNA, primers, SYBR Premix Ex Taq II (TaKaRa), etc. Primers were 5'-GAATTCGGATGCAGAGCTTC-3', 5'-GACATGCTGCGGTGTTTTC-3' for EGFR; 5'-CCATCTTCCTGCTG CTGTAACTG -3', 5'-GCCTTCTCTGCCAACTGTCC-3' for MTHFR. The EGFR gene amplification fold was normalized to H441 cells, and ⩾2 fold was regarded as EGFR gene amplification.
  - **FISH assay:** Vysis EGFR/CEP7 FISH probe (Abbott) and Accessory Kit (DAKO) were used. The presence of tight EGFR gene clusters (red signals) in ⩾10% tumor cells was defined as EGFR gene amplifications.
- **Gene mutation test for EGFR, PIK3CA, K-ras and B-raf:** Genomic DNA was extracted using the QIAamp Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Hotspots in exon 19, 20, 21 of EGFR gene, exon 2 and 3 of K-ras gene, exon 11 and 15 of B-raf gene, and exon 9 and 20 of PIK3CA gene were screened at Genewiz Company by using ABI3730XL sequence analyzer.
- **PDX model development and anti-tumor efficacy study:** Fresh tumor specimen was subcutaneously implanted into NOD-SCID mice (P0), and subsequent mouse to mouse passages were made in additional NOD-SCID or nude mice once the tumor size reached 300~500 mm^3. After several consecutive in vivo passages, the tumors (⩾P3) were used to evaluate the anti-tumor efficacy of EGFR inhibitor Epitinib.

Results

A. Profiling summary of 43 esophagus tumors from Chinese patients

Squamous	EGFR high expression	EGFR amp.	EGFR mut	K-ras/B-raf/PIK3CA mutation
39/43 (91%)	30/43 (70%)	3/43 (7%)	15/43 (35%)	0/34 (0%)

- Three samples with EGFR gene amplification were identified by qPCR and FISH.
- No mutation was found in the 43 samples for K-ras (G12, G13, Q61), B-raf (G464, V600) or PIK3CA (E542, E545 and H1047).

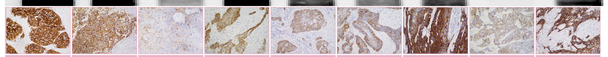


Individual profiling data of 43 esophagus tumors from Chinese patients

ID	Histopathology	EGFR expression by IHC						EGFR amp. (Normalized to H441)	EGFR mutation
		0 (%)	1+ (%)	2+ (%)	3+ (%)	Hscore	Categorical score		
ESO2T0040	squamous	0	10	10	80	270	3+	0.5	No
ESO2T0046	squamous	0	0	0	100	300	3+	2.5	No
ESO2T0048	small cell	100	0	0	0	0	0	0.4	No
ESO2T0059	squamous	100	0	0	0	0	0	0.6	No
ESO2T0060	squamous	0	10	60	30	220	2+	0.6	Q787Q
ESO2T0061	squamous	0	0	70	30	230	2+	0.5	Q787Q
ESO2T0068	squamous	0	0	20	80	280	3+	0.5	Q787Q
ESO2T0071	squamous	0	0	0	100	300	3+	0.5	No
ESO2T0085	squamous	0	0	10	90	290	3+	1.3	No
ESO2T0093	squamous	0	70	30	0	130	1+	1.0	No
ESO2T0096	adeno.	0	80	20	0	120	1+	1.4	No
ESO2T0128	squamous	0	0	0	100	300	3+	0.6	No
ESO2T0137	sarcoma	100	0	0	0	0	0	0.8	No
ESO2T0139	squamous	0	0	10	90	290	3+	5.5	No
ESO2T0142	squamous	0	20	10	70	250	3+	1.4	No
ESO2T0151	squamous	0	0	10	90	290	3+	1.0	Q787Q
ESO2T0180	squamous	0	50	40	10	160	1+	1.4	No
ESO2T0185	squamous	0	10	10	80	270	3+	0.7	No
ESO2T0256	squamous	0	10	80	10	200	2+	1.1	No
ESO2T0258	small cell	100	0	0	0	0	0	0.9	No
ESO2T0260	squamous	0	10	10	80	270	3+	0.3	No
ESO2T0269	squamous	0	10	70	20	210	2+	0.6	Q787Q
ESO2T0278	squamous	0	0	20	80	280	3+	1.2	Q787Q
ESO2T0279	squamous	0	50	20	30	180	1+	0.9	K745K
ESO2T0281	squamous	0	70	20	10	140	1+	0.3	Q787Q
ESO2T0282	squamous	10	0	0	90	270	3+	0.4	Q787Q
ESO2T0289	squamous	0	20	60	20	200	2+	0.3	Q787Q
ESO2T0294	squamous	100	0	0	0	0	0	0.2	No
ESO2T0313	squamous	0	10	60	30	220	2+	0.4	Q787Q
ESO2T0315	squamous	0	40	0	60	220	3+	1.3	No
ESO2T0330	squamous	100	0	0	0	0	0	0.5	Q787Q
ESO2T0335	squamous	0	10	20	70	260	3+	0.5	H850L
ESO2T0340	squamous	0	10	60	30	220	2+	0.6	No
ESO2T0345	squamous	0	20	60	20	200	2+	0.8	No
ESO1T0326	squamous	0	0	0	100	300	3+	23.3	No
ESO1T0327	squamous	0	30	30	40	210	3+	1.7	No
ESO1T0412	squamous	0	70	20	10	140	1+	1.8	Q787Q
ESO1T0472	squamous	0	20	70	10	190	2+	1.2	No
ESO1T0474	squamous	0	0	70	30	230	2+	1.8	No
ESO1T0586	squamous	0	30	70	0	170	2+	1.9	Q787Q
ESO1T0768	squamous	0	0	10	90	290	3+	1.3	No
ESO1T0773	squamous	30	70	0	0	70	1+	0.8*	No*
ESO1T0781	squamous	0	0	20	80	280	3+	1.6	No

H score =0x(% at 0)+1x(% at 1+)+2x(% at 2+)+3x(% at 3+); *, the sample was from PDX model, not from the patient sample.

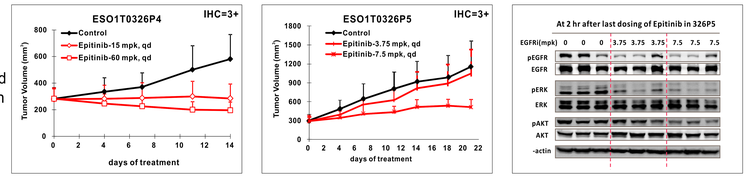
B. Correlation between EGFR expression and the sensitivity to Epitinib in PDX models

PDX model	#326	#327	#412	#472	#474	#586	#768	#773	#781
EGFR WB									
EGFR IHC									
IHC score	3+	3+	1+	2+	2+	2+	3+	1+	3+
H score	300	210	140	190	230	170	290	70	280
Others	EGFR Amp.	FGFR1 OE		PIK3CA Mut					
TGI% at 7.5 mpk	74.6				27.5				49.0
TGI% at 30 mpk	98.2* 129.1**	29.6	TBD	85.7	70.2	TBD	TBD	5.7	80.7

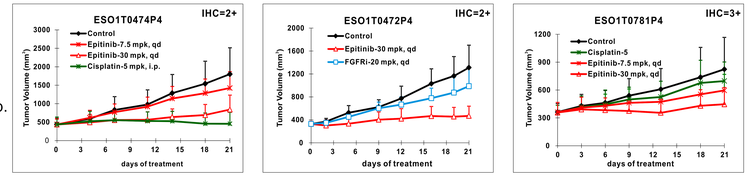
*, TGI at 15 mg/kg; **, TGI at 60 mg/kg

- The sensitivity of PDX models to Epitinib correlated well with EGFR IHC scoring, except for ESO1T0327, in which FGFR1 high expression was found.

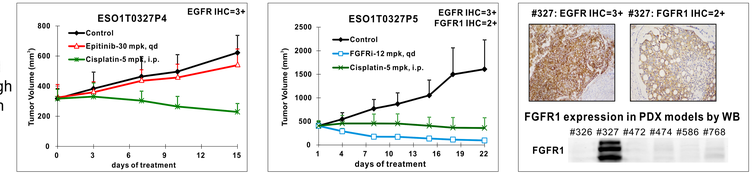
EGFR amp. and high expression



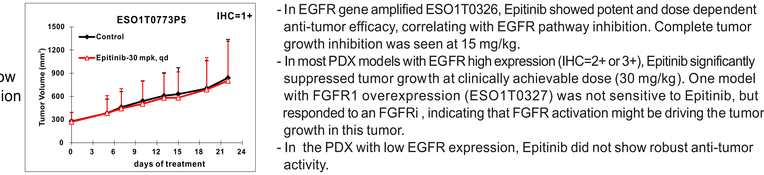
EGFR high expression without amp.



EGFR and FGFR1 high expression



EGFR low expression



- Epitinib was orally administered once daily to nude mice bearing estabilised PDX models:
  - In EGFR gene amplified ESO1T0326, Epitinib showed potent and dose dependent anti-tumor efficacy, correlating with EGFR pathway inhibition. Complete tumor growth inhibition was seen at 15 mg/kg.
  - In most PDX models with EGFR high expression (IHC=2+ or 3+), Epitinib significantly suppressed tumor growth at clinically achievable dose (30 mg/kg). One model with FGFR1 overexpression (ESO1T0327) was not sensitive to Epitinib, but responded to an FGFRi , indicating that FGFR activation might be driving the tumor growth in this tumor.
  - In the PDX with low EGFR expression, Epitinib did not show robust anti-tumor activity.

Conclusions

- High expression of EGFR was frequently found in Chinese esophagus cancer.
- EGFR inhibition resulted in potent anti-tumor effect in multiple patient derived esophagus cancer models carrying EGFR amplifications and/or high expression, suggesting that anti-EGFR agents might bring clinical benefits to esophagus cancer patients with abnormal EGFR activation.
- Aberrant FGFR signaling might lead to resistance to EGFR inhibitors.

References

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