The Predictive Value of Fragile-site Associated Tumor Suppressor (FATS) Gene Expression on the Sensitivity of Cisplatin in Advanced Non-Small Cell Lung Cancer

Rabea AM[1], Atef A[2], Desouky E[3], Ashmawy A[2], Khorshid O[1], Gaber A[1], Gaafar R[1]

[1] Medical oncology department, National Cancer Institute, Cairo University
[2] Tumor Biology department, National Cancer Institute, Cairo University
[3] Statistics and Epidemiology department, National Cancer Institute, Cairo University

Received: 5 January 2022
Accepted: 13 February 2022

Abstract:

Background Cisplatin based chemotherapy regimens have been the standard of care for the treatment of advanced stage non-small cell lung cancer, however not all patients respond adequately to the treatment. We therefore assessed the expression of Fragile-site Associated Tumor Suppressor (FATS) gene and the response to cisplatin.

Patients and methods: A prospective longitudinal study will be conducted in NCI (National Cancer Institute) medical oncology department in outpatient setting, in the period between June 2012 and July 2014. The study included 70 patients with pathologically proven advanced (stage IIIB and IV) non-small cell lung cancer treated with Cisplatin-based regimens. The primary end point was progression free survival (PFS) while the secondary end points were overall survival (OS) and overall response rate (ORR).

Results: Seventy patients were assessed and only 57 patients were evaluated for the trial. Ten patients achieved partial response while 6 patients achieved stable disease and rest had a progressive disease. Median PFS was 4.1 months (95% CI 2.1-6.2) in the low expression group which was not statistically different compared to 3.8 months (95% CI 3.3-4.3) in the high expression group (p= 0.442) while the median OS was statistically significant in high expression group [6.9 months (95% CI 5.4-8.3) compared to low expression group [4.5 months (95% CI 3.7-5.3)] (p= 0.031).

Conclusion: The expression of the FATS gene can have an implication on the response to cisplatin and the overall survival. This opens the opportunity that FATS gene can be used as a predictive marker for NSCLC patients receiving cisplatin-based chemotherapy.

Keywords: NSCLC, FATS, Cisplatin

Authors Information:
Ahmed Magdy Rabea
Medical oncology department, National Cancer Institute, Cairo University
email: Ahmed.rabea@live.com

Ahmed Atef
Tumor Biology department, National Cancer Institute, Cairo University
email: Ahmed.atef@nci.cu.edu.eg

Eman Desouky
Statistics and Epidemiology department, National Cancer Institute, Cairo University
email: dr.emandesouky@yahoo.com

Abeer Ashmawy
Tumor Biology department, National Cancer Institute, Cairo University
email: abeermanci@yahoo.com

Ola Khorshid
Medical oncology department, National Cancer Institute, Cairo University
email: olakhorshid@yahoo.com

Ayman Gaber
Medical oncology department, National Cancer Institute, Cairo University
email: gaberayman@hotmail.com

Rabab Gaafar
Medical oncology department, National Cancer Institute, Cairo University
email: rabab.gaafar@gmail.com

Corresponding Author:
Ahmed Magdy Rabea
Medical oncology department, National Cancer Institute, Cairo University
email: Ahmed.rabea@live.com

Introduction:

Lung cancer is the leading cause of cancer incidence and mortality in both sexes according to the GLOBOCAN (Global Cancer Incidence, Mortality and Prevalence) reported in 2020 [1]. With all the new available medications for lung cancer ranging from chemotherapy to immune checkpoint inhibitors, platinum salts are still the backbone of the first-line regimens in non-small cell lung cancer (NSCLC), albeit this fact, we have encountered many patients who do not respond adequately to Cisplatin-based regimens.

A chromosomal fragile site is a specific heritable point on a chromosome that forms a gap or constriction and could lead to break when the cell is exposed to partial replication stress [2]. Fragile-site associated tumor suppressor (FATS) gene is a newly identified candidate tumor suppressor whose gene locus is frequently deleted in spontaneous tumors. Chromosomal fragile sites are loci that are prone to

gaps or breaks within metaphase chromosomes. Common fragile sites (CFSs) are observed in all humans and constitute a component of normal chromosome structure. CFSs have an important role in chromosome instability [3]; they are associated with sister chromatid exchange hotspots, viral integration sites and sites of deletion, amplification, and translocation in various cancers [4]. CFSs are now being implicated as regions of high genomic instability associated with cancer [5]. The genomic instabilities created by the defects in cell-cycle checkpoints resulted and initiated tumorigenesis [6].

A Chinese study stated that FATS gene plays an important role in the stabilization of p21 gene which is an important cell cycle checkpoint gene preserving the genome integrity. FATS gene locus is susceptible to DNA damage induced by DNA damaging agents, and the deletion frequency of FATS gene is dramatically increased in tumor genome triggered by DNA damage [7]. These processes are under the control of unmutated p53 gene, which is a major tumor suppressor gene that is frequently mutated in most of the malignancies [8]. Down regulation or silence of FATS has been observed in mouse lymphoma, human ovarian cancer, and multiple cancer cell lines [9].

A more recent Chinese study has concluded that in patients who underwent surgical excision of the tumor, showed high expression of FATS gene in the mRNA and responded better to Cisplatin based chemotherapy [10].

Patients and Methods:

70 patients were included in the study with pathologically proven stage IV NSCLC of all pathological types (i.e., Adenocarcinoma, Squamous cell carcinoma and undifferentiated large cell carcinoma) from the period of June 2012 to July 2014 at the National Cancer Institute, Cairo University. Informed consent was approved by The Institutional Review Board (IRB) committee at the National Cancer Institute, Cairo University and was signed for the approval to use the biopsied tissue. As a control, 30 histologically proven normal healthy lung tissue from the same patients were included.

All patients received Gemcitabine 1000 mg/m2 days 1 and 8 with Cisplatin 75 mg/m2 day 1 only. Supportive measures were given including antiemetics, gastric protection and symptomatic treatment together with hydration. Chemotherapy toxicity was evaluated with each cycle as per CTCAE ver. 4 and dose reduction was done for any grade 3 or 4 toxicity.

For the survival analysis, PFS was measured from the start of chemotherapy till disease progression while OS was measured from the date of starting chemotherapy till the last date the patient was seen or deceased.

The tissues were obtained from the paraffin block. 70 samples were collected, however only 57 samples could be done, and the rest were discarded due to technical issues or consumed with no available tumor tissue.

To determine the expression level of FATS gene, RNA extraction where performed, followed by complementary DNA synthesis and qPCR.

The concentration and purity of the RNA was determined using UV-spectrophotometer Gene-ray (Biometera, Germany) and all samples ratio was in the range of 1.7 to 2.

Complementary DNA was done using Quanti-tech reverse transcriptase kit (Qiagen, Germany) according to manufacture instructions. All incubated samples were done in thermal cycler (Eppindorf, Germany) and the cDNA yielded was diluted 1:2. To confirm the quality of cDNA all samples were tested against housekeeping gene (GAPDH) first. All samples were good for FATS profiling.

GAPDH forward primer:
5'-GGGAAGGTGAAGGTCGGAGTC-'3
GAPDH reverse primer:
5'-TTCTCAGCCTTGACGGTGCCAT-'3
FATS forward primer:
5'-CATTCACATTCCTGGCTGGAGTTA-'3
FATS reverse primer:
5'-CCTCTTGCTGCTTCCAGAAAATACT-'3

For qPCR the kit used was KAPA SYBR® FAST qPCR Kit Master Mix (2X) Universal (kappa Biosystems, USA)

Statistical Methods:

the data management and statistical analysis were performed using Statistical Package for Social Sciences (SPSS) version 21.

Numerical data are summarized by using the means and standard deviations or ranges and medians. Categorical data were summarized as percentages and comparisons between the 2 groups with respect to normally distributed numeric variables were done using the t-test. Abnormally distributed numeric variables were compared by Mann-Whitney test. For categorical variables, differences were analyzed with Chi square test and Fisher's exact test when appropriate. Kaplan and Meier procedure was used to estimate the progression free rates and overall survival. Comparisons between the different prognostic factors were done using the Log-rank test.

Patients lost to follow-up were censored on last known alive date. All P-values are two-sided. P-values < 0.05 were considered significant.

Results:

The study included 70 patients who presented to the National Cancer Institute, Cairo University during the period of June 2012 to July 2014 with a median follow up period of 6 months. Table (1) summarizes the 70 patients' characteristics regarding their age, gender, pathology type, smoking history, performance status and disease stage.

The response rate was evaluated for the 70 included patients, 44 patients (62%) had progressive disease while 26 patients had clinical benefit, 13 patients (18.5%) had regressive disease and 13 patients (18.5%) had stable disease (Table 2).

Table 1: Summary of patients' characteristics of the 70 patients included in this study

Characteristics	Number (%)
Age:	
Range	30-74
Mean± SD	54.8±7.9
Gender:	
Male	64 (91.4)
Female	6 (8.8)
Pathological subtypes:	
Adenocarcinoma	34 (48.5)
Squamous cell carcinoma	14 (20)
Undifferentiated Large cell	22 (31.5)
Stage:	
IIIB	3 (4.3)
IV	67 (95.7)
PS (ECOG):	
I	60 (85.7)
II	10 (14.3)
Smoking:	
Yes	63 (90)
No	7 (10)

Table 2: Response rates of the 70 patients included in this study

Type of response	Number	Percentage
Progressive disease	44	62
Best response (SD+PR+CR)	26	38
Partial response	13	18.57
Stable disease	13	18.57
Complete response	0	0

In this study, none of the evaluated clinical variables were found to be statistically significant in correlation with the response to chemotherapy (Table 3).

The 57 evaluable patients in the study, FATS expression was correlated with the site of metastasis and there was no statistical significance even when grouping the most aggressive sites (brain and bone) in comparison to other sites of metastasis (Table 4).

Table 3: Correlation between different clinical variables and response to chemotherapy

Factors	Non-responsive n=51	Responsive n=19	P value
Age (yrs.)			
Mean±SD	54.2±9.1	56.1±5.5	0.420
Gender			
Male	47(72.1)	17(27.9)	0.777
Female	4(66.7)	2(33.3)	
Smoking			
No	6(71.4)	2(28.6)	0.822
Yes	48(72.0)	14(28.0)	
PS			
I	42(69.0)	18(31.0)	0.217
II	9(88.9)	1(11.1)	
Stage			
III	3(100)	0(0)	0.169
IV	43(75.0)	14(25.0)	
Pathology type			
Adenocarcinoma	26(74.2)	8(25.8)	0.785
Squamous cell carcinoma	9(63.6)	5(36.4)	
Undifferentiated Large Cell	16(73.7)	6(26.3)	
Need for 2nd line			
No	38(76.1)	11(23.9)	0.232
Yes	13(61.9)	8(38.1)	

Table 4: FATS expression in relation to site of metastasis among the 57 evaluable patients in the study

	Brain & Bone			Other sites			
	Median	Minimum	Maximum	Median	Minimum	Maximum	**p-value**
FATS expression	0.0089	0	0.2597	0.0168	0	0.6518	0.695

FATS gene expression was correlated to the best overall response (CR + PR + SD) versus progressive disease (PD) and there was a statistically significantly higher median FATS expression in patients with progressive disease (p= 0.031) (Table 5).

Table 5: Correlation between FATS expression and response to chemotherapy (ORR vs PD) among the 57 evaluable patients in the study

Factors	Overall response n=31	Progressive disease n=26	P value
FATS expression			
Median(range)	0.010(0-0.652)	0.035(0-0.260)	0.031

Even when comparing the FATS expression in each clinical response criteria, there was a significant response benefit in patients lower FATS expression with responding disease (RD) (p= 0.02) (Table 6).

Table 6: FATS expression in relation to each response category among the 57 evaluable patients in the study

	Regressive disease	Stable disease	Progressive disease	P-value
Median FATS expression	0.005	0.035	0.010	0.020

57 patients could be assessed for the FATS gene expression by RT-PCR with a median value of 0.002. It was noted that the FATS gene expression was markedly reduced in all the specimens in comparison to normal lung tissue. According to the median value, the patients have been divided into low expression group (≤0.002) and high expression group (>0.002).

The 2-year PFS rates in both the high expression and the low expression group were in the range of 4 months and were not statistically significant (p= 0.422) (Table 7, figure 1).

Table 7: Correlation between FATS expression and PFS among the 57 evaluable patients in the study

FATS Expression	Number	Median PFS (months) (95%CI)	P-value
≤0.002	11	4.1(2.1-6.2)	0.422
>0.002	46	3.8(3.3-4.3)	

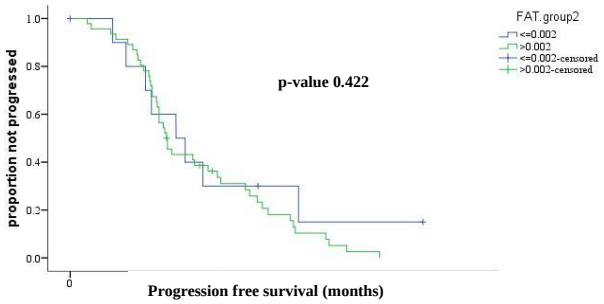

Figure 1: Correlation between FATS expression and PFS among the 57 evaluable patients in the study

On the other hand, the OS showed a statistical difference between patients with high FATS expression and patients with low FATS expression, with a median OS of 6.9 months in the high expressors group versus 4.5 months in the low expressors group (p= 0.031) (table 8, figure 2).

Table 8: Correlation between FATS expression and OS among the 57 evaluable patients in the study

FATS Expression	Number	Median OS (months) (95%CI)	P-value
≤0.002	11	4.5(3.7-5.3)	0.031
>0.002	46	6.9(5.4-8.3)	

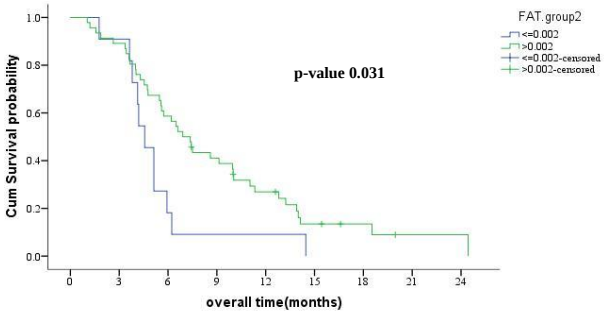

Figure 2: Correlation between FATS expression and OS among the 57 evaluable patients in the study

Discussion:

Our study showed that FATS gene expression was markedly reduced in comparison to the normal lung tissue specimens examined, the results were like the initial trial for the correlation of FATS gene expression in lung cancer by Li et al [7] and the study correlating

the FATS gene expression and response to Cisplatin based chemotherapy by Tian et al [10]. In our study, the median FATS gene expression was 0.002 which was the cutoff value that we considered above is high expression and below is low expression which was lower when compared to Tian et al's study [10] which showed that the median level was 0.042 and indicated the involvement of FATS gene in NSCLC, this difference might be attributed to more advanced stages included in our study and demographic differences. It has also been proven that the FATS gene was markedly reduced in lung, breast, osteosarcoma, ovarian and cervical cell lines; faintly expressed in colon cancer cell lines as stated by Li et al [7].

As for the relation of FATS gene expression and its relation to the clinical variables, we found that there was no statistical significance between high and low expression FATS and demographics which comes in agreement with Tian et al [10] where he also found no statistical significance between FATS gene expression and the clinical variables.

Regarding the response rates, the overall response rates (SD+RD) were statistically significant in the high expression group versus the low expression group (p-value 0.031).

In our study, the median PFS was 4 months in both the high and low FATS expression groups, and it was not statistically significant (p-value 0.422).

Scagliotti et al [11] have shown in NSCLC randomized clinical trials evaluating platinum doublets that the median survival time ranged from 7.4 to 10.1 months. In our study, the maximum OS was 24 months, and the minimum OS was 3.7 months. The calculated OS was statistically significant (p-value 0.031) in the high FATS expression group with a median survival of 6.9 months versus 4.5 months for the low expression group. We observed that the PFS and OS outcomes in our study were lower than other studies which may be attributed to late diagnosis with high tumor burden, continuation of smoking and exposure to pollutants.

As FATS is a fragile site gene with important functions in maintaining genomic stability, our study suggests that FATS gene has a potential application in predicting response to chemotherapy benefit for those drugs triggering DNA damage response mainly Gemcitabine and Cisplatin. Identification of molecular markers with predictive value in response to cisplatin treatment therefore may be helpful for the development of individualized treatment strategies to further improve efficacy and minimize side effects.

Conclusion:

We recommend to further study the FATS gene expression in larger studies to validate its predictive value. Quantitative measurement of FATS mRNA may be a new approach with potential application in personalized therapy of NSCLC which will allow for proper choice of treatment for newly diagnosed advanced stage NSCLC, minimizing the side effects of cisplatin chemotherapeutic agent leading to further improvement of the quality of life for our patients. It can also be used further in the diagnosis of many cancers as it has been proven that its level has been markedly reduced in many cancers cell lines.

References:

1. Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today
2. Sutherland GR, Hecht F. Fragile Sites on Human Chromosomes. New York and Oxford: Oxford University Press 1985.
3. Durkin SG, Glover TW. Chromosome fragile sites. Annu Rev Genet 2007;41:169–192
4. Burrow AA, Williams LE, Pierce LC, et al. Over half of breakpoints in gene pairs involved in cancer-specific recurrent translocations are mapped to human chromosomal fragile sites. BMC Genomics 2009; 10, 59
5. Buttel I, Fechter A, Schwab M. Common fragile sites and cancer: targeted cloning by insertional mutagenesis. Ann NY Acad Sci 2004; 1028: 14–27.
6. Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. Nature 2004, 432: 316–323
7. Li Z, Zhang Q, Mao J, et al. An HDAC1-binding domain within FATS bridges p21 turnover to radiation-induced tumorigenesis. Oncogene 2010; 29:2659-2671
8. Vousden KH, Lane DP. p53 in health and disease. Nature Rev Mol Cell Biol 2007; 8:275-283
9. Zhang X, Zhang Q, Zhang J, et al. FATS is a transcriptional target of p53 and associated with antitumor activity. Mol Cancer 2010; 9:244
10. Tian Y, Zhang J, Yan Set al. FATS expression is associated with cisplatin sensitivity in non-small cell lung cancer. Lung Cancer 2012; 76(3):416-22
11. Scagliotti GV, De Marinis F, Rinaldi M, et al. Phase III randomized trial comparing three-platinum-based doublets in advanced non-small-cell lung cancer. J Clin Oncol 2002; 20:4285-4291.