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EGFR Somatic Mutations in Lung Tumors: Radon Exposure and Passive-smoking in Former- and Never-smoking U.S. Women

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Abstract

Background—Lung cancer patients with mutations in *EGFR* tyrosine kinase have improved prognosis when treated with *EGFR* inhibitors. We hypothesized that *EGFR* mutations may be related to residential radon or passive tobacco smoke.

Methods—This hypothesis was investigated by analyzing *EGFR* mutations in seventy lung tumors from a population of never and long-term former female smokers from Missouri with detailed exposure assessments. The relationship with passive-smoking was also examined in never-smoking female lung cancer cases from the Mayo clinic.

Results—Overall, the frequency of *EGFR* mutation was 41% [95% Confidence Interval (CI): 32-49%]. Neither radon nor passive-smoking exposure was consistently associated with *EGFR* mutations in lung tumors.

Conclusions—The results suggest that *EGFR* mutations are common in female, never-smoking, lung cancer cases from the U.S, and *EGFR* mutations are unlikely due to exposure to radon or passive-smoking.

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Conflict of interest statement
No potential conflicts of interest were disclosed.

Keywords

EGFR mutations; never-smokers; lung cancer; radon; passive-smoking; second hand smoke; tobacco smoke

Introduction

Among never-smokers, lung cancer is the seventh leading cause of cancer death. A large proportion of lung cancer in never-smokers remains unexplained by established environmental risk factors. However, radon and passive-smoke exposure were associated with lung cancer in never-smokers in several studies (for review (1)).

Lung cancer has a poor prognosis overall, but small molecule inhibitors of *EGFR* result in improved survival in some patients. Therapeutic response correlates with somatic mutations in the *EGFR* gene. Those mutations are inversely correlated with cigarette smoking and more frequently observed in never-smokers (for review (2)). We investigated the possibility that residential radon or passive-smoking was associated with the presence of *EGFR* mutations in lung tumors in two populations of female never and long-term former smokers.

Methods

Study Populations

The Missouri Women's Health Study case series included Caucasian lung cancer cases nested within a case-control study of never- and former-smoking women (3-4). Lung cancer patients from the Mayo Clinic were described previously (5). Cases were limited to Caucasian women to ensure comparability to the Missouri study.

EGFR Mutation Analysis

Missouri Women—DNA previously isolated from microdissected tumor samples (4), available from 105 of 132 samples, was used for *EGFR* mutation analysis in the Laboratory of Human Carcinogenesis. Due to evaporation, the majority of DNA samples (74% or 78/105) were reconstituted using 10 μl of Tris-EDTA buffer (pH 7.5). PCR amplification was performed as a 50 μl reaction including 2 μl DNA stock solution, 1.25 U of Native Pfu DNA polymerase (Stratagene), 1X Pfu buffer, 300 nM forward and reverse primers for either exon 19 or exon 21 of EGFR; primers were identical to those reported previously (6). Amplification was performed using the following conditions: 95°C for 5 minutes followed by 40 cycles of 96°C for 45 sec, 58°C for 1 min, and 72°C for 1 min; a terminal extension cycle of 5 min at 72°C was included. If initial PCR reactions failed to amplify, a second PCR amplification reaction was performed using 5 μl of a 1:10 dilution of the PCR reaction mixture. Samples that failed the first series of amplifications were re-amplified using a second aliquot of genomic DNA. Overall, thirty-two (30%) of 105 genomic samples failed to amplify. DNA sequencing was performed as per manufacturer's instructions on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) by NCI DNA MiniCore Facility using the PCR amplification primers. Forward and reverse sequences were 100% concordant.

Mayo Clinic—*EGFR* mutations were analyzed at the Mayo Clinic as part of oncogene mutation screening using the OncoCarta™ Panel v1.0 (Sequenom, San Diego, CA) on the Sequenom MassArray Genetic Analysis platform following manufacturer's protocol. Data analysis was performed using MassArray Typer Analyzer 4.0 software (Sequenom, San Diego, CA). Performance of the assay was evaluated against a panel of lung tumor samples

and cell lines with previously identified mutations. EGFR gene mutation data was available for all 73 cases from the Mayo clinic study.

Statistical Analysis

Samples with incomplete sequencing data for *EGFR* (e.g. failed amplification at one or more exons) were considered missing. The Mayo Clinic study had mutation data on additional EGFR exons compared to the Missouri study. Cases with mutations in exons other than 19 and 21 were considered wild-type for *EGFR* (N=3). Results were similar when they were considered mutant (data not shown).

Never-smokers were defined as persons who had either smoked < 100 cigarettes or not used any tobacco products during their lifetimes. To examine association of any exposure to passive-smoking in the Missouri study, categories of exposure (< 21, 21-52, and >52 pack-years) were combined and compared to 0 pack-years. Former-smokers in the Missouri study abstained from tobacco for at least 15 years prior to interview (3). In the Mayo Clinic Study, passive-smoke dosimetry included both adult and/or in childhood exposures. Data analysis was performed by SAS version 9.1 (SAS Institute, Cary, NC) using two-sided tests in the Laboratory of Human Carcinogenesis.

Results

Twenty-four of the Missouri cases (34%; 95% CI: 23% - 47%) and thirty-four of the Mayo clinic cases (47%; 95% CI: 35% - 59%) had mutations detected in exons 19 or 21 of *EGFR* (Table 1). Overall, the mutation frequency was 41% (95% CI: 32% - 49.0%).

While there was a difference in the quartiles of radon exposure associated with *EGFR* mutation (P=0.01), this was not significant when exposure was dichotomized at the median (P=0.14, Fisher's Exact Test) and no difference was observed when considering radon as a continuous measure (P=0.16) and there was no evidence for a dose-response relationship with radon exposure (Table 2).

In the Missouri Women's Health Study cases, there was an inverse association of *EGFR* mutations with any exposure to passive-smoke, but no clear dose response relationship was observed with passive-smoke exposure quantified in pack-years. In the Mayo clinic population, no association was observed between *EGFR* mutations and adult exposure, childhood exposure, or any exposure to passive-smoke (Table 2).

Discussion

Our data do not support the hypothesis that radon exposure contributes to mutations in *EGFR*. The mutation frequency appeared elevated at low-dose exposure and diminished at higher exposure levels, but we noticed a similar trend with *TP53* mutations (4). The relationship of radon with lung cancer risk is thought to be linear (1), so our inverse trend between radon dose and *EGFR* mutations probably occurred by chance.

We observed an inverse association of passive-smoke exposure with *EGFR* mutations in lung tumors in the Missouri study, but this finding failed to replicate in the Mayo Clinic never-smoker patient cohort. Previously, an inverse association with passive-smoke exposure as an adult or in childhood was observed (7). However, another study linked long-term exposure to passive-smoking with excess *EGFR* mutations (8).

In conclusion, we observed a high frequency of *EGFR* mutations in lung tumors from never-smoking and long-term former-smoking women in the U.S., but no association between *EGFR* mutations with passive-smoking or residential radon exposure.

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Table 1

EGFR Gene Mutations in Never and Former Smoking Lung Cancer Patients

Patient ID	Histology	EGFRexon	Mutation Type	Codon(s)	Nucleotide Change	Amino Acid Change
Missouri Women						
2	adenocarcinoma	19	Deletion	del Glu746-Ala750		
6	other/mixed	21	Point	858	CTG>CGG	Leu>Arg
48	small cell carcinoma	21	Point	866	GAG>GAT	Glu>Asp
54	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
56	adenocarcinoma	19	Deletion	del Glu746-Ala750		
57	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
60	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
69	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
71	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
72	adenocarcinoma	19	Deletion	del Glu746-Ala750		
74	adenocarcinoma	21	Point	833	TTG>TTT	Leu>Phe
76	adenocarcinoma	19	Deletion plus insertion	del Leu747-Pro753 (ins Ser)[a]		
78	adenocarcinoma	19	Deletion plus insertion	del Leu747-Ala750 (ins Pro)[a]		
81	adenocarcinoma	19	Point	743	GCT>ACT	Ala>Thr
88	adenocarcinoma	21	Point (silent)	858 (silent)	CTG>CTT	Leu>Leu
95	adenocarcinoma	19	Deletion	del Glu746-Ala750		
104	adenocarcinoma	21	Point	864	GCG>GTG	Ala>Val
105	adenocarcinoma	19	Deletion	del Glu746-Ala750		
126	adenocarcinoma	19	Deletion	del Glu746-Ala750		
129	bronchioloalveolar carcinoma	19	Deletion	del Glu746-Ala750		
134	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
135	bronchioloalveolar carcinoma	19	Deletion plus insertion	del Glu746-Ser752 (ins Val)[a]		
136	adenocarcinoma	21	Point (silent)	848 (silent)	CCG>CCT	Pro>Pro
139	other/mixed	19	Deletion	del Ser752 -Ile759		

Mayo Clinic Study

Patient ID	Histology	EGFRexon	Mutation Type	Codon(s)	Nucleotide Change	Amino Acid Change
	adenosquamous					
921901995	carcinoma	21	Point	858	CTG>CGG	Leu>Arg
921901997	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
921902006	bronchioloalveolar carcinoma	21	Point	858	CTG>CGG	Leu>Arg
921902011	adenocarcinoma	19	Deletion	del Glu746-Ala750		
921902017	adenosquamous carcinoma	21	Point	858	CTG>CGG	Leu>Arg
921902018	adenocarcinoma bronchioloalveolar	19	Deletion	del Glu746-Ala750		
921902021	carcinoma	19	Deletion plus insertion	del Leu747-Pro753 (ins Ser)[a]		
921902023	adenocarcinoma	19	Deletion	del Glu746-Ala750		
921902024	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
921902032	adenocarcinoma	19	Deletion	del Glu746-Ala750		
921902033	adenosquamous carcinoma	19	Deletion	del Glu746-Ala750		
921902049	adenocarcinoma	19	Deletion	del Glu746-Ala750		
921902055	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
921902063	adenocarcinoma	19	Deletion plus insertion	del Leu747-Ala750 (ins Pro)[a]		
921902067	adenocarcinoma	19	Deletion	del Glu746-Ala750		
921902070	adenocarcinoma	19	Deletion	del Glu746-Ala750		
921902071	adenocarcinoma	19	Deletion plus insertion	del Glu746-Thr751 (ins Val)[a]		
921902084	adenocarcinoma	19	Deletion	del Glu746-Ala750		
921902086	bronchioloalveolar carcinoma	19	Deletion	del Glu746-Ala750		
921902118	adenosquamous carcinoma	21	Point	858	CTG>CGG	Leu>Arg
921902119	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
921902121	adenocarcinoma	19	Deletion	del Glu746-Ala750		
921902132	adenocarcinoma	19	Deletion	del Glu746-Ala750		
921902140	adenocarcinoma	19	Deletion	del Glu746-Ala750		
921902148	adenocarcinoma	19	Deletion	del Glu746-Ala750		

Patient ID	Histology	EGFRexon	Mutation Type	Codon(s)	Nucleotide Change	Amino Acid Change
921902154	adenocarcinoma	19	Deletion	del Glu746-Ala750		
921902157	squamous cell carcinoma	19	Deletion	del Glu746-Ala750		
921902160	squamous cell carcinoma	19	Deletion	del Glu746-Ala750		
921902161	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
921902163	adenocarcinoma	19	Deletion plus insertion	del Leu747-Ala750 (ins Pro)[a]		
921902171	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
921902172	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
921902175	adenocarcinoma	21	Point	858	CTG>CGG	Leu>Arg
921902187	adenocarcinoma	19	Deletion	del Glu746-Ala750		

[a]ins, Insertion of Amino Acid in parentheses

Table 2

Association of EGFR mutations with Patient Charactersitics

	Missouri Women EGFR					Mayo Clinic Study EGFR				
	−Mutation N=46		+Mutation N=24			−Mutation N=39		+Mutation N=34		
	N	%	N	%	p-value					p-value
Histological Subtype										
Adenocarcinoma[a]	34	*74*	19	*80*	0.98[b]	28	*72*	29	*85*	0.28[b]
Bronchioloalveolar carcinoma	4	*9*	2	*8*		4	*10*	3	*9*	
Squamous cell carcinoma	2	*4*	0	*0*		3	*8*	2	*6*	
Small cell carcinoma	1	*2*	1	*4*		0	*0*	0	*0*	
Other	5	*11*	2	*8*		4	*10*	0	*0*	
Age (years)										
median, IQR	76	*64-79*	66	*61-78*	0.24[c]	68	*57-75*	72	*67-79*	0.06[c]
missing	3		1							
Passive-Smoke										
No exposure	21	*47*	17	*74*	0.04[b]	10	*32*	4	*15*	0.22[b]
Any exposure	24	*53*	6	*26*		21	*68*	22	*85*	
Missing	1		1			8		8		
Passive-Smoke (Pack-years)										
No exposure	21	*47*	17	*74*	0.08[b]	*nd*		*nd*		
< 21	10	*22*	3	*13*						
21-52	11	*24*	1	*4*						
>52	3	*7*	2	*9*						
Missing	1		1							
Passive-Smoke (Adult Exposure)										
No exposure	*nd*		*nd*			11	*35*	8	*31*	0.78[b]
Any exposure						20	*65*	18	*69*	

	Missouri Women EGFR					Mayo Clinic Study EGFR				
	−Mutation N=46		+Mutation N=24			−Mutation N=39		+Mutation N=34		
	N	%	N	%	p-value					p-value
Missing						8		8		
Passive-Smoke (Child Exposure)										
No exposure	*nd*		*nd*			22	*71*	13	*50*	0.17[b]
Any exposure						9	29	13	50	
Missing						8		8		
Radon Exposure (Bq/m^3)										
4.8 - 33.3	13	*30*	5	*23*	0.01[b]	*nd*		*nd*		
35.2 - 55.5	5	*11*	9	*41*						
56.2 - 82.7	9	*20*	6	*27*						
> 82.7	17	*39*	2	*9*						
missing	2		2							
Radon Exposure (Bq/m^3)										
median, IQR	63.7	*30.5-94.1*	46.5	*37.0-57.4*	0.16[c]	*nd*		*nd*		
missing			*2*							

nd, not determined

[a] Five of the adenocarcinomas in the Mayo Clinic Study were adenosquamous histology.

[b] Fisher's Exact test

[c] Wilcoxon two-sample test

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