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Review

Mutations of the epidermal growth factor receptor in non-small cell lung cancer – Search and destroy

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ABSTRACT

The targeting of the ATP binding pocket of the epidermal growth factor receptor (EGFR) tyrosine kinase, by the small molecule drugs gefitinib and erlotinib, represents a promising new therapeutic strategy in non-small cell lung cancer. However, it is now apparent that only a subset of patients responds to such treatment. Two publications in early 2004 reported the presence of activating mutations in the EGFR tyrosine kinase gene conferring exquisite sensitivity to these drugs. Several publications have since reported prospective data consistent with this finding. This brief review summarises the mutation data from 15 such studies in terms of mutation frequency by clinicopathological features and correlation with response to tyrosine kinase inhibition. A new paradigm for the routine detection of such mutations is needed to facilitate patient selection for treatment and further studies.

1. Introduction

Lung cancer is the leading cause of cancer-related death in the developed world. Recent statistics demonstrate that the disease accounts for around 183,000 fatalities across Europe with over 33,000 of these in the UK [1,2]. There is an estimated incidence of 1.2 million new cases each year worldwide [3]. Non-small cell lung cancer (NSCLC) makes up 80% of all primary pulmonary tumours. For advanced NSCLC median survival remains poor at 7.9 months and only approximately one third of patients survive for one year or more despite conventional combination chemotherapy. Moreover, such therapy is associated with considerable toxicity [4,5]. Clearly, there is a requirement for the development of more effective systemic treatment with fewer side-effects.

Recent unravelling of the molecular aspects of cancer biology and improvements in biotechnology and clinical pharmacology have permitted the development of novel drugs against specific targets associated with oncogenic drive as part of the new therapeutic armoury. An example is inhibition of the epidermal growth factor receptor (EGFR) system. To date, two classes of drugs have been licensed in this area: small molecule tyrosine kinase inhibitors (TKI) and monoclonal antibodies to the EGFR. The clinical studies however, show only modest numbers of clinical responders. One area of current research focuses upon the identification of distinguishing factors between those who derive benefit from those who do not – infor-

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mation which will aid patient selection. The last twelve months have seen a flood of publications documenting mutations within the EGFR gene and their correlation with tumour sensitivity to tyrosine kinase inhibition. In this brief review, we aim to bring together and summarise the mutation data from 15 scientific reports and discuss the possible impact on the therapy of NSCLC.

2. EGFR as a molecular target for cancer therapy

The erb-B (HER) family is one of several known receptor tyrosine kinase systems involved in cellular signalling [6,7] and comprises four different receptors of which EGFR (erbB1) was the first discovered; the other family members are erbB2 (HER2), erbB3 (HER3) and erbB4 (HER4). The structure of EGFR consists of an extracellular ligand-binding domain, a transmembrane domain and a cytoplasmic component containing a tyrosine kinase entity. The erb-B family of receptors transduce effects of approximately eleven different ligands [8]. Extracellular ligand-binding induces conformational changes resulting in the formation of receptor dimers and oligomers. Tyrosine kinase phosphorylation then ensues and heralds second messenger signal transduction via mitogen activated protein kinase (MAPK), phosphatidylinositol 3 kinase (PI3K) and other pathways, effecting cellular processes such as proliferation, angiogenesis and survival [9].

EGFR protein overexpression, sometimes as a consequence of gene amplification, has been demonstrated in several epithelial tumour types and sometimes predicts a more aggressive phenotype and worse prognosis [10]. Other mechanisms of aberrant EGFR activation include overexpression of receptor ligands, loss of negative regulation pathways and activating mutations [11]. The phenomenon of mutations leading to such disruption of EGFR signalling is considered an example of 'oncogene addiction' by tumours and as such provides a point of therapeutic attack [12,13]. EGFR is expressed in up to 93% of NSCLC patients in whom approximately 45% show overexpression [14,15]. Based on these observations, EGFR targeted therapeutics is an attractive option for exploration in this disease.

3. EGFR tyrosine kinase inhibition

Currently, two tyrosine kinase inhibitors are in clinical use: gefitinib and erlotinib [16,17]. These small-molecule agents compete with and prevent the binding of adenosine triphosphate (ATP) at the ATP-binding region within the EGFR tyrosine kinase thereby inhibiting tyrosine residue phosphorylation and signalling. Trials of single agent use in chemotherapy-refractory NSCLC patients have demonstrated response rates of 9–18% and median survival ranged from 6.7 to 8.4 months [18–21]. Furthermore, a large phase III study of erlotinib monotherapy showed a significant survival benefit compared to placebo (6.7 months vs 4.7 months; $P = 0.001$) [20]. A similarly designed study using gefitinib however, did not show any survival gain over placebo (ISEL study results, AstraZeneca EAP update letter) and exemplified the unpredictable nature of therapeutic response. Large well-conducted phase III trials of platinum-based chemotherapy in combination with either gefitinib or erlotinib were conducted with hope of demonstrating synergy. Unfortunately, neither TKIs showed statistically significant benefit over chemotherapy alone [22–25]. These disappointing data caused much speculation as to the importance of the dose level, treatment schedule and possibility of drug antagonism. However, in these studies, many of the patients who derived benefit from receiving a TKI, had both dramatic and durable responses. Subsequently, mutations of the EGFR have been described and appear to predict for tumour sensitivity to TK inhibition.

4. EGFR mutations are common in East Asian female non-smokers

The middle of 2004 saw two separate reports published describing mutations within the TK domain of the EGFR in NSCLC [26,27]. Since then, a multitude of data has emerged from different groups from around the world. The EGFR gene consists of 118 kbp in 28 exons; the TK domain is encoded within exons 18–21. Fifteen studies [26–40] have reported mutational analyses of this area in over 3000 cases of NSCLC (Table 1). The overall mutation rate observed in unselected cases of NSCLC in this data set is 16.7% but differs widely according to ethnicity: broadly, those of East Asian origin have a higher prevalence compared to Caucasians (30.6% vs 7.6%, $P < 0.0001$). Mutation rates are also higher in non-smokers (34.8% vs 7.8%, $P < 0.0001$) and women (26.4% vs 9.3%, $P < 0.0001$). The reasons for this are as yet unclear; though it is suggested that this may be a form of NSCLC whose tumorigenesis is independent of tobacco smoking and historically, females have smoked less than men and this gap was especially wide in regions of the Pacific rim until recently [41,42]. These demographic distinctions are in keeping with observations within clinical trials of TKIs in that East Asian female non-smokers are more likely to carry mutations and respond to treatment [43]. Furthermore, mutations appear at a higher frequency in NSCLC patients with adenocarcinoma (23.2%) or bronchioloalveolar carcinoma (BAC) (17.9%) histology when compared to others (2.2%).

5. Mutation hotspots in the TK domain

Although a variety of different mutations are seen spanning the entire EGFR TK domain, 89% reside in exons 19 and 21 (Fig. 1). Of note are two particular mutations: deletion of amino acids 746–750 in exon 19 (del(746–750)) and leucine to arginine substitution at 858 in exon 21 (L858R) which together account for approximately 66% of all alterations (Fig. 2). Interestingly, the majority of other mutations seen in exon 19 are variants of del(746–750) and approximately 80% involve the deletion of the ELREA amino acid sequence. This amino acid sequence flanks the ATP binding cleft of the TK – the very same target area of the TKIs: gefitinib and erlotinib. The L858R mutation lies close to a highly conserved region: the DFG motif. The precise influence of such mutations on drug-binding is as yet unclear and the possibility exists that this may vary at least subtly depending on the mutation type.

Matched normal lung tissue was available in the majority of patients and sequencing has confirmed that the observed mutations within the tumour are somatic. In most cases mutations are heterozygous suggesting a dominant effect leading to gain of function and tumour cell survival advan-

Table 1 – Clinicopathological and gefitinib response data from 15 publications [26–40]

Study	Lynch	Paez	Pao	Han	Marchetti	Shigematsu	Mitsudomi	Huang	Kosaka	Qin	Sasaki	Soung	Yang	Cappuzzo	Cortes-Funes	Total[§]	%
No. of total cases	34	128	131	90	860	617	59	117	277	41	118	153	219	89	83	3016	
No. of unselected cases	9	119	96	90	860	519	59	117	277	41	118	153	219	89	83	2849	
Gef−																	
Mutations	2	16	11	0	39	120(10)	0	39	111	10	16	30	26	0	0	420	16.7
WT	23	103	85	0	821	399(88)	0	62	166	31	86	123	193	0	0	2092	83.3
Gef+																	
Mutations with PR/CR	(8)	5	(12)	11	0	0	26	7	0	0	5	0	0	9	6	69	76.7[a]
WT with PR/CR	(1)	0	(5)	10	0	0	3	2	0	0	0	0	0	NS	6	21	23.3
Mutations with SD	0	0	0	4	0	0	1	0	0	0	0	0	0	0	4	9	13.2
WT with SD	0	0	0	23	0	0	2	0	0	0	0	0	0	0	34	59	86.8
Mutations with PD	0	0	0	2	0	0	5	1	0	0	1	0	0	6	0	15	12.3[a]
WT with PD	0	4	(18)	40	0	0	19	6	0	0	10	0	0	NS	28	107	87.7
Not evaluable							3								5	8	
Histology[§]																	
Mutations in adeno.	0(5)	15(4)	(10)	13	17	114	32	46	110	7	19	26	25	10	10	444	23.2[b]
Total adeno. In sample	7(5)	70(7)	NS	55	289	289	50	81	224	17	63	69	164	50	42	1470	
Mutations in BAC	2(3)	0(1)	(2)	1	22	0	0	0	0	0	0	0	0	2	0	27	17.9[c]
Total BAC in sample	15(4)	NS(1)	NS	10	86	0	0	3	0	0	2	0	0	8	0	124	
Other (than adeno.)	0(0)	1(0)	NS	2	0	6	1	1	1	3	3	4	1	3	0	26	2.2[b,c]
Total other in sample	3(0)	49(0)	NS	25	485	230	9	33	53	24	53	84	55	31	41	1175	
No. with multiple mutations[§]	0	4	1	1	0	3	4	7	6	1	0	0	1	0	0	28	
Sex[§]																	
Mutations in male patients	(3)	6(2)	(6)	5	18	48	14	20	41	6	8	13	12	7	3	201	9.3[d]
Total male patients	(3)	74(3)	NS	54	748	348	32	60	159	30	87	111	134	58	58	1953	
Mutations in female patients	(5)	10(3)	(6)	12	21	72	19	27	70	4	14	17	14	8	7	284	26.4[d]
Total female patients	(6)	45(6)	NS	36	112	171	27	57	118	11	31	42	85	31	25	791	
Sex NS	2															2	
Smoking[§]																	
Mutations in smokers*	(3)	NS	4(3)	6	16	35	13	6	35	4	7	5	14	9	4	158	7.8[e]
Total smokers*	(3)	NS	81(NS)	47	745	353	31	NS	162	20	NS	99	185	76	63	1862	
Mutations in non-smokers	(5)	NS	7(9)	11	23	85	20	41	76	6	15	25	12	6	6	333	34.8[e]
Total non-smokers	(6)	NS	15(NS)	43	115	166	28	NS	115	21	NS	54	34	13	20	624	
Mutation rates by ethnicity[§]																	
USA	2 of 25	1 of 61	11 of 85			11 of 80							21 of 191	15 of 89			11.5
Japanese		15 of 58				71 of 263	33 of 59		111 of 277		22 of 118						32.5
Korean				17 of 90								30 of 153					19.3
Italian					39 of 860								5 of 28				5.0
Spanish															10 of 83		12.0
Chinese										10 of 41							24.4
Taiwanese						32 of 93		47 of 117									37.6
Australian						6 of 83											7.2
Total Caucasians																121 of 1585	7.6[f]

(*continued on next page*)

Table 1 – (*continued*)

Study	Lynch	Paez	Pao	Han	Marchetti	Shigematsu	Mitsudomi	Huang	Kosaka	Qin	Sasaki	Soung	Yang	Cappuzzo	Cortes-Funes	Total[§]	%[f]
Total East Asians																388 of 1269	30.6[f]
Other																	
Cell lines sequenced	108	4								7							
Cell lines with mutations	0	1								0							
Other tumours sequenced						243											
Other tumours mutations						0											

( ) numbers in parentheses indicate selected cases and are not included in totals. Gef−, not on gefitinib therapy. Gef+, on gefitinib therapy. WT, wildtype EGFR. SD, stable disease. PR/CR, partial response/complete response. PD, progressive disease. adeno., adenocarcinomas including adenocarcinoma with BAC features. BAC, bronchoalveolar carcinoma. * smokers: includes former smokers. § includes only unselected cases. a, b, c, d, e, f $P < 0.0001$ (2-sided comparisons using Fisher's exact test for 2×2 contingency tables). NS, not specified in paper. NA, not applicable.

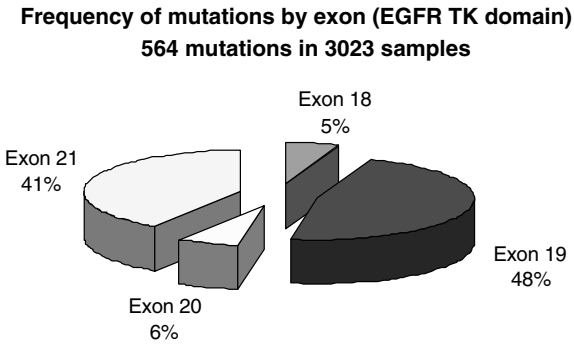

Fig. 1 – Pie-chart of frequency of mutations by exon.

tage. Although most studies focused on aberrations within the TK domain, DNA sequencing of the entire 28-exon EGFR locus in ~200 patients [31] has failed to reveal other mutations or alterations including the large deletions in the extracellular domain found at high frequency in glioblastoma multiforme tumours [44].

6. Sensitivity to TK inhibition

Patients with NSCLC responsive to gefitinib or erlotinib are more likely to harbour mutations than not (76.7% vs 23.3%). However, 12.3% of those NSCLCs carrying mutations still progress on TKIs underlining the imperfect correlation. Furthermore, there are probably alternative mechanisms conferring sensitivity given that not all responders carry mutations; this is exemplified by the fact that 86.8% of those achieving stable disease possess wild-type EGFR. It may be that some other mechanism exists whereby a cytostatic effect is achieved as opposed to a cytotoxic one.

In vitro studies have confirmed enhanced growth inhibition by gefitinib in mutant-EGFR transfected mammalian cells [26,28] and a mutant EGFR containing cancer cell line [27]. With regards to functional activity, EGFR mutant cells have both heightened and prolonged responses to EGF stimulation [26]. Furthermore, siRNA studies eliminating the mutant EGFR protein leads to extensive apoptosis confirming its role as an oncogene [45]. Three separate groups have reported the finding of a secondary mutation in exon 20 bestowing tumour resistance to gefitinib [46–48]. This T790M amino acid substitution has been observed in a total of six patients and one cancer cell line (H1975) and seemingly heralds resistance to gefitinib and erlotinib therapy. It is thought that position 790 lies within the ATP/drug binding cleft causing steric hindrance to drug-docking. This observation matches that of tyrosine kinase inhibition using imatinib for gastrointestinal stromal tumours and chronic myeloid leukaemia; here, imatinib resistance is also observed but can be overcome with newer generation small molecules [49]. Hence there is hope for gefitinib and erlotinib resistant NSCLC.

7. Other mutations within the EGFR system

Apart from within the EGFR TK domain, few other mutations have been observed in other receptors of the EGFR family.

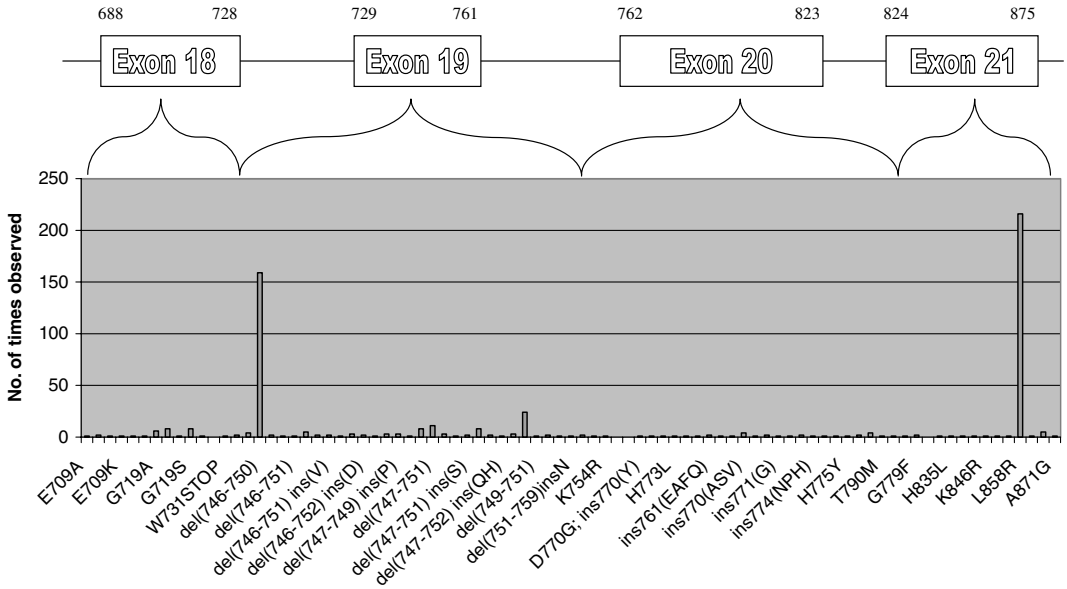

Fig. 2 – Mutations and their frequency in 3023 patients.

1011 assorted tumour samples and cell lines have been sequenced for HER2 mutations amongst which only 20 have been found; 17 of these are from NSCLC tumours (overall HER2 mutation rate in NSCLC: 2%). Notably, all but one of these mutations are exon 20 in-frame duplications or insertions [50,51]. To date no data is available as to correlation of HER2 mutations with tumour characteristics or response to therapy of any kind. Interestingly, the short span of exon 20 in which these mutations lie closely correspond to positions at which several EGFR mutations reside. HER3 and HER4[1] have not been studied to date in this regard. It will be fascinating to elucidate how different receptor mutations might affect function, dimerisation and drug sensitivity. The other focus of observed mutations within the EGFR signal transduction pathway in lung cancers lies in K-ras. K-ras activation is a downstream signalling event; K-ras mutations have been found in 3.9–8% of NSCLCs, mostly in adenocarcinomas (12–29%). Interestingly, mutations in K-ras and EGFR very rarely occur concomitantly [30,31,34,37,52], suggesting that either mutation alone is perhaps sufficient for cellular survival advantage and lung tumorigenesis and may have a different aetiology. Crucially, evidence exists suggesting that K-ras mutation confers resistance to gefitinib and erlotinib [46,52] allowing further enrichment of treatment groups. Mutations in other components of this signalling cascade have not yet been observed in NSCLC.

8. Do EGFR TK mutations occur in other epithelial tumours?

DNA sequencing of 243 other epithelial tumours in one study, has shown no EGFR TK mutations [31]. However, there has been a recent report of mutations in 3 of 41 (7.3%) cases of squamous cancer of the head and neck; interestingly, all were exon 19 deletions of residues 746–750, the most common deletion found in NSCLC; however, all 3 patients were male smokers [53]. It will be important to determine if cases of this genotype may also be sensitive to gefitinib or erlotinib therapy.

9. Mutations in NSCLC: search and destroy

Clearly, the presence of EGFR TK mutations partially correlates with tumour sensitivity to currently available TKIs. Such mutations have also been shown to confer resistance to conventional chemotherapy. There are therefore major implications to therapeutic decision-making – perhaps the earlier use of gefitinib or erlotinib is justified in such patients. It is just a matter of time before patients with newly-diagnosed NSCLC and their relatives will insist upon EGFR genotyping to aid clinical management. Several methods for the detection and screening of mutations have been suggested including direct sequencing of PCR products with single-strand conformation polymorphism (SSCP) [30], LightCycler PCR assay [36], PNA-LNA PCR [54] and PCR with RFLP [55]. However, outside of an academic centre with such resources and expertise, most institutions will find routine screening for mutations a major challenge and timely and expensive to set up. Perhaps, screening for mutations should only apply to patients with predictive clinical and histological features i.e. East Asian origin, female sex, non-smoking status and adenocarcinoma/BAC. However, such a strategy may miss ~10% of patients who would obtain significant benefit from TKI treatment. It is also arguable that all patients should be offered TKIs since there exists a cohort who benefit from symptom improvement without tumour response [56,57]. Others argue that, it is still worthwhile identifying those with activating mutations since TK inhibition may be preferred to chemotherapy earlier on in treatment. Furthermore, in these selected patients studies might be performed using TKIs in

[1] Since publication, HER4 mutations have been described in human cancers [59].

the adjuvant and even neoadjuvant setting, following the recent exciting results with trastuzumab [58]. Prospective trials of different methods and protocols of mutation detection are awaited in the hope of improving outcomes in lung cancer. With keen anticipation, NSCLC with EGFR mutations shall be sought and destroyed.

Conflict of interest statement

None declared.

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