

***EGFR* activating aberration occurs independently of other genetic aberrations or telomerase activation in adenocarcinoma of the lung**

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Received February 13, 2007; Accepted March 22, 2007

Abstract. The prognosis of lung cancer remains poor, and biological heterogeneity is largely responsible, especially in adenocarcinoma. We previously found that only one third of non-small cell lung cancer (NSCLC) but most small cell lung cancer (SCLC) tissues have strong telomerase activity, representing the difference in the history of multiple clonal selections. To reveal the genes differentially involved in telomerase activation mechanisms, we analyzed the relationship between common genetic aberrations and telomerase activity in 83 lung cancer tissues. We found that half (7 of 14) of lung adenocarcinomas with high telomerase activity showed neither *TP53* nor *RBI* deletion, while all squamous cell carcinomas and SCLCs with high telomerase activity showed loss of heterozygosity of at least one, if not both, of these suppressor oncogenes, indicating that these genetic aberrations are not required in activation of telomerase in a unique subset of adenocarcinoma. Furthermore, whereas the aberrations in *TP53*, *RBI* and 1p34-pter were mutually related in 42 adenocarcinoma tissues, *EGFR* aberrations showed no relationship to either of them. These findings indicate that *EGFR* activating aberrations occur independently of other common genetic aberrations or telomerase activation mechanisms in lung adenocarcinoma, and that the distinct subset of lung adenocarcinoma with high telomerase activity without any common genetic aberrations may possibly have arisen from a telomerase-positive or telomerase-competent normal cell.

Introduction

Lung cancer is a major cause of morbidity and mortality worldwide. In the last decade significant progress has been made in the understanding of the molecular mechanisms which are responsible for human cancer development and progression. A large number of chromosomal alterations and genetic defects have been identified in lung cancer (1,2). Among them, inactivating aberrations of *TP53* and *RBI* genes and loss of heterozygosity (LOH) at various loci are frequently observed in lung cancer. Repeated clonal selection of cancer cells accumulating such genetic aberrations is considered to promote cellular immortalization concomitant with telomerase activation (3), encouraging novel anticancer strategies targeting telomere and telomerase (4-6). However, we recently found that carcinogenesis is not necessarily a prerequisite for activation of telomerase in lung cancer, and that the non-cancerous bronchial epithelia with telomerase expression have a predisposition to develop lung cancer (7). Then, to clarify the activation mechanism of telomerase in lung cancer, we here examined the relationship between genetic aberrations and activation of telomerase. As common genetic aberrations in lung cancer we analyzed *TP53*, *RBI*, and 1p34-pter deletions, which we previously found to be related with alteration with telomere length in lung cancer (8). As adenocarcinoma-characteristic aberrations, we investigated activating aberrations of *EGFR* and *KRAS*.

EGFR, a 170-kDa (1,186 amino acids) membrane-bound protein encoded by 28 exons spanning nearly 190,000 nucleotides on chromosome 7p12, is one member of the ErbB family of receptor tyrosine kinases which include four closely related receptors: HER-1/ErbB1, HER-2/*neu*/ErbB2, HER-3/ErbB3 and HER-4/ErbB4 (9-11). EGFR consists of an extracellular domain that binds EGF, transforming growth factor alpha (TGF- α), and other growth factors, a short transmembrane region, and an intracellular tyrosine kinase domain. Ligand binding leads to homodimerization or heterodimerization of EGFR with other receptors then recruiting intracellular signaling proteins; converting extracellular signals to intracellular signal transduction events (12,13). EGFR-activated pathway includes Akt and signal transducer and activator of

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Key words: *EGFR*, telomerase, *TP53*, *RBI*, lung adenocarcinoma

transcription (STAT) cascades important for cell survival and mitogen-activated protein kinase (MAPK) pathway, which induces cell proliferation (11,14). Dysregulation of these pathways appears to play a crucial role in oncogenesis and progression of solid tumors via effects on cell-cycle progression, inhibition of apoptosis, induction of angiogenesis, and promotion of tumor-cell motility and metastasis (15,16). *EGFR* is known to be overexpressed in 40-80% of non-small cell lung cancers (NSCLCs) (15), suggesting that the activation of the *EGFR* signal pathway plays an important role in tumorigenesis of lung cancers. However, its activating aberrations, mutations and amplification, were revealed to be found solely in adenocarcinomas, giving them biologically distinct characteristics (10,17,18). *EGFR* mutants may lead to abnormally sustained responses to EGF, activation of different downstream signaling pathways, and induction of distinct patterns of phosphotyrosine proteins (19-21). These *EGFR* mutants as well as amplified *EGFR* may transduce augmented survival signals, on which the cancer cells become dependent showing high sensitivity to gefitinib (9,20,22). However, little is known of the relationship with activation of telomerase in cancer, and here we demonstrate that only *EGFR* activating aberrations occur independently of other molecular events common in lung cancer, including telomerase activation, while others relate with each other, and that there is a distinct subset in lung adenocarcinoma in which telomerase is highly activated without commonly known genetic aberrations.

Materials and methods

Tumor samples. We examined the relationship between various genetic aberrations and telomerase activity in 83 primary lung cancer tissues surgically resected from chemotherapy-naïve patients. We used corresponding adjacent non-cancerous lung tissue samples as control. All tissues were provided by the Departments of Pathology and Molecular and Internal Medicine, Hiroshima University between 1991-1996, with their pathological stages assessed according to the International Staging System (Hermanek and Sobin, 1987) and telomerase activity and chromosome 1p deletion mapping previously reported (3,23). They consisted of 42 adenocarcinomas (male 24, female 18), 30 squamous cell carcinomas (male 28, female 2), 7 small cell carcinomas (male 6, female 1), and 4 adeno-squamous carcinomas (male 4, female 0). Written informed consent was obtained from all patients before surgery, and this study was approved by our Institutional Ethics Committee.

Telomerase activity and genetic aberrations. Among the genetic aberrations analyzed in this study, we previously reported the telomerase activity level (3) and 1p34 deletion mapping (23) for all samples, and loss of heterozygosity (LOH) of *TP53* and *RB1* genes and point mutation of the *KRAS* gene (8) for some of them (Table I). The remaining samples were examined by the same procedure for this study. Briefly, genomic DNA was extracted from frozen tissues using proteinase K and SDS. LOH of the *TP53* gene was assessed by PCR-based restriction fragment length polymorphisms (PCR-RFLPs) for the *AccII* site in exon 4 and the *ApaI* site in intron 7, polyacrylamide gel electrophoresis for a 16-nt insertion polymorphism in intron 3, and fragment analysis for

a 5-nucleotide microsatellite polymorphism in intron 1 and a dinucleotide microsatellite polymorphism at the 5'-flanking region of *TP53* (X61505). LOH of the *RB1* gene was assessed by PCR-RFLP for the *XbaI* site in intron 17 and fragment analysis for microsatellite markers in introns 2, 4 and 20 (8). LOH at 1p34-pter was assessed by deletion mapping at 12 loci (23) and mutations at the *KRAS* codons 12 and 13 were detected by designed PCR-RFLP (8) and confirmed by direct sequencing in the present study.

***EGFR* mutation analysis by direct sequencing.** *EGFR* mutation was examined by direct sequencing for exons 18, 19, 20 and 21. The primers used for PCR were as follows (24): Exon 18, 18F: 5'-AGCATGGTGAGGGCTGAGGTGAC-3'; 18R: 5'-ATATACAGCTTGCAAGGACTCTGG-3'. Exon 19, 19F: 5'-CCAGATCACTGGGCAGCATGTGGCACC-3'; 19R: 5'-AGCAGGGTCTAGAGCAGAGCAGCTGCC-3'. Exon 20, 20F: 5'-GATCGCATTTCATGCGTCTTCACC-3'; 20R: 5'-TTGCTATCCCAGGAGCGCAGACC-3'. Exon 21, 21F: 5'-TCAGAGCCTGGCATGAACATGACCCTG-3'; 21R: 5'-GGTCCCTGGTGTGTCAGGAAAATGCTGG-3'. Approximately 100 ng of genomic DNA was amplified by PCR in 20 μ l reaction mixture containing 200 μ M each dNTP, 1.5 mM $MgCl_2$, 0.5 mM each primer, and 1 U of AmpliTaqGold™ (Perkin-Elmer, NJ, USA). PCR amplification protocol was performed using a Program Temp Control System PC-818 (Astec, Fukuoka) for one initial cycle of 15 min at 95°C, and 40 cycles of denaturation at 95°C for 60 sec, annealing at 66°C for 60 sec, and strand elongation at 72°C for 60 sec were performed to amplify DNA fragments followed by a final elongation at 72°C for 5 min. The PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide. The PCR products were sequenced using BigDye™ Terminator v1.1 chemistry (Applied Biosystems, Foster City, CA, USA) in combination with an ABI PRISM™ 310 genetic analyzer (Applied Biosystems). *EGFR* mutation loci were identified according to GenBank accession number NM_005228. All mutations were confirmed by at least two independent PCR amplifications.

***EGFR* amplification analysis by real-time PCR.** *EGFR* copy number was determined by TagMan™ quantitative real-time PCR system with TagMan™ Universal PCR master mix and the ABI PRISM™ 7900HT sequence detection system (Applied Biosystems). The quantitative real-time *EGFR* amplification was performed with 20 ng of genomic DNA in a 10- μ l reaction mixture using a 384-well reaction plate (Applied Biosystems) and 2 sets of primers and probe: for *EGFR* and each control gene. The primer and MGB-probe sequences and final concentrations optimized for *EGFR* amplification analysis were as follows: *EGFR* (25), *EGFR*-F: 5'-CAATTGCCAGTTAACGTCTTCCTT-3', 300 nM; *EGFR*-R: 5'-TTTCTCACCTTCTGGGATCCA-3', 900 nM; *EGFR*-probe: FAM-TCTCTCTGTCATAGGGAC, 200 nM. *TFRC* as different chromosome (chr. 3) control (26), *TFRC*-F: 5'-GCCAATGAGGTCTGAAATGGA-3', 300 nM; *TFRC*-R: 5'-GGCCTTATTCCTGCAATCAACA-3', 900 nM; *TFRC*-probe: VIC-CTTCTGCTGGATAAAATGAGGTTCAA, 200 nM. *COG5* as chromosome 7 control (25), *COG5*-F: 5'-TGGAAGATGATGCACAAGATATATTCA-3', 300 nM; *COG5*-R: 5'-CCAA

Table I. List of genetic aberrations in primary lung cancers with adenocarcinoma component.

									EGFR		
No.	Gender	Age	Stage	Tel ^a	TP53	RBI	1p34 ^a	KRAS	Mut/Amp	ex20	IVS1 repeat no.
Adenocarcinoma											
1	F	61	I	Low	Hetero	Hetero	Hetero	w/w	w/w	w/w	20/22
2	M	69	I	Low	Hetero ^a	Hetero ^a	Hetero	w/w ^a	w/w	w/w	18/20
3	M	75	I	Low	Hetero	Hetero	Hetero	w/w	w/w	w/w	15/16
4	M	62	I	Low	Hetero ^a	Hetero ^a	Hetero	w/w ^a	w/w	w/v	16/20
5	M	66	I	Low	Hetero ^a	Hetero ^a	Hetero	w/w ^a	Amp	w/v	20
6	F	67	I	Low	Hetero	Hetero	Hetero	w/w	del746-750	w/w	16/20
7	F	67	II	Low	Hetero ^a	Hetero ^a	Hetero	w/w ^a	w/w	w/w	20
8	M	76	IIIA	Low	Hetero	Hetero	Hetero	w/w	w/w	w/v	16/20
9	M	61	IIIA	Low	Hetero	Hetero	Hetero	w/w	w/w	w/w	20
10	M	60	IIIB	Low	Hetero	Hetero	Hetero	w/w	w/w	w/w	20
11	F	64	IV	Low	Hetero ^a	Hetero ^a	Hetero	w/w ^a	w/w	w/v	16/20
12	M	56	IV	Low	Hetero ^a	Hetero ^a	Hetero	w/w ^a	w/w	w/v	20
13	F	57	IIIA	Low	Hetero	Hetero	LOH	w/w	w/w	w/w	17/20
14	M	60	IV	Low	Hetero ^a	Hetero ^a	LOH	w/w ^a	w/w	w/w	20
15	F	65	IV	Low	Hetero ^a	Hetero ^a	LOH	w/w ^a	del746-750	w/w	20
16	F	62	I	Low	Hetero ^a	LOH ^a	LOH	w/w ^a	del747-51	w/v	20
17	F	60	I	Low	NI	Hetero	Hetero	w/w	w/w	w/v	20/22
18	M	48	I	Low	NI ^a	Hetero ^a	LOH	w/w ^a	w/w	w/w	20
19	F	51	IIIa	Low	NI	Hetero	Hetero	w/w	w/w	w/v	15/20
20	F	57	I	Low	LOH	Hetero	LOH	w/w	w/w	w/w	17/20
21	M	58	IV	Low	LOH ^a	Hetero ^a	Hetero	w/w ^a	L858R,Amp	w/v	17/20
22	F	56	IV	Low	LOH ^a	Hetero ^a	Hetero	G12C/w	w/w	w/v	20
23	M	66	IV	Low	LOH ^a	Hetero ^a	Hetero	G12C/w	w/w	w/v	20
24	F	72	IIIA	Low	LOH ^a	LOH ^a	Hetero	w/w ^a	w/w	w/w	20/22
25	M	67	I	Low	LOH	LOH	LOH	w/w	del746-50	w/w	20
26	M	77	I	Low	LOH ^a	LOH ^a	LOH	w/w ^a	w/w	w/v	20/21
27	M	64	I	Low	LOH	LOH	LOH	w/w	w/w	w/w	20/23
28	M	62	IIIA	Low	Hetero ^a	LOH ^a	LOH	w/w ^a	w/w	w/w	16
29	F	78	I	High	Hetero	Hetero	Hetero	w/w	w/w	w/w	16/20
30	M	69	I	High	Hetero	Hetero	Hetero	w/w	w/w	w/v	15
31	M	62	I	High	Hetero	Hetero	Hetero	w/w	w/w	w/w	20
32	M	75	I	High	Hetero ^a	Hetero ^a	Hetero	w/w ^a	w/w	w/w	20
33	F	62	II	High	Hetero	Hetero	Hetero	w/w	del746-50,Amp	w/v	20
34	F	69	IIIA	High	Hetero	Hetero	Hetero	w/w	w/w	w/v	15
35	F	77	IV	High	Hetero ^a	Hetero ^a	Hetero	w/w ^a	w/w	w/v	20
36	F	80	I	High	Hetero ^a	LOH ^a	Hetero	w/w ^a	del747-51	w/w	20
37	F	59	IIIA	High	LOH	Hetero	Hetero	w/w	w/w	w/w	16/20
38	M	76	IIIA	High	LOH	Hetero	LOH	w/w	Amp	w/w	20
39	M	55	I	High	LOH ^a	LOH ^a	LOH	w/w ^a	ins773-6,Amp	w/w	20
40	M	68	II	High	LOH	LOH	LOH	w/w	w/w	w/w	20
41	M	66	IV	High	LOH	LOH	Hetero	w/w	w/w	v/v	20
42	M	50	IIIB	High	LOH ^a	LOH ^a	LOH	w/w ^a	w/w	w/v	16/20
Adenosquamous carcinoma											
43	M	75	IIIA	Low	Hetero ^a	Hetero ^a	Hetero	w/w ^a	w/w	w/w	16/20
44	M	42	I	Low	NI	LOH	Hetero	G13D/w	w/w	w/v	20
45	M	75	IV	Low	LOH ^a	LOH ^a	Amp	w/w ^a	w/w	w/w	20
46	M	69	IIIA	High	LOH	LOH	LOH	w/w	w/w	w/w	20

^aPreviously reported: all tel, telomerase activity (3,23); all 1p34, deletion mapping from 1p34 to pter (23); and cases of TP53, LOH in the TP53 gene; RB1, LOH in the RB1 gene; and KRAS, mutations in codon 12 or 13 (8). Others are the present results.

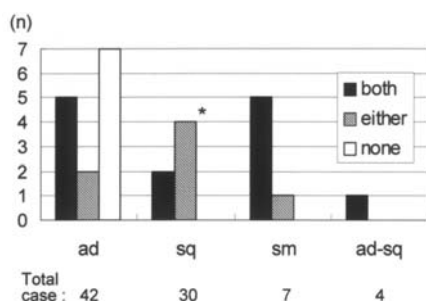


Figure 1. Frequencies of loss of heterozygosity (LOH) in *TP53* and *RB1* genes in lung cancer with high telomerase activity. ad, adenocarcinoma (total cases: n=42); sq, squamous cell carcinoma (n=30); sm, small cell carcinoma (n=7); ad-sq, adenosquamous carcinoma (n=4). *Including one non-informative case for *RB1* with LOH for *TP53*.

CTAACAGGTCAAATTAAACAAACA-3', 300 nM; COG5-probe: VIC-CCAAAAAAGCCAGATTATGA, 50 nM. The relative genomic copy number was calculated using the comparative threshold method. Threshold cycle (Ct) for each gene was determined using thermocycler software and the average of 3 independent Cts was calculated. *EGFR* amplification was defined when the differences in the threshold amplification cycles between target gene (*EGFR*) and different chromosome control gene (*TFRC*) were ≥ 0.8 . This cut-off value was based on the differences in the adjacent non-cancerous lung tissues.

Genotyping of *EGFR* intron 1 polymorphism and assessment of allelic imbalance by fragment analysis. Genomic DNA of tumor tissues was subjected to PCR using a FAM-labeled forward primer (5'-FAM-GGGCTCACAGCAAACCTTCTC-3') and a non-labeled reverse primer (5'-AAGCCAGACTCGCTCATGTT-3') (27) with annealing temperature at 55°C. The PCR products were analyzed by ABI PRISM 310 genetic analyzer and GeneScan™ software (Applied Biosystems). (CA)_n repeat number was determined by the fragment size and confirmed by direct sequence of the representative sample. Allelic imbalance was defined when the peak height ratio of heterozygous bands was <50% or >200% of expected ratio calculated from the normal cells.

Statistical analysis. For statistical analysis, the relationship between *EGFR* activating aberrations and adenocarcinoma subgroups or other genetic changes was assessed by χ^2 or Fisher's exact tests, as appropriate. Differences of $P < 0.05$ were considered statistically significant.

Results

Telomerase activity and LOH in *TP53* and *RB1*. Among the 83 primary lung cancers, high telomerase activity [6 μ g of protein extract expressed comparable TRAP signals with those using 100 or more immortal cancer cells in TRAP assay (3)] was found in 14 of 42 adenocarcinomas (33.3%), 6 of 30 squamous cell carcinomas (20.0%), 6 of 7 small cell carcinomas (85.7%), and 1 of 4 adenosquamous carcinomas (25.0%), and LOH of *TP53* and *RB1* was found in 15 (35.7%) and 11 (26.2%) adenocarcinomas, 21 (70.0%) and 12 (40.0%) squamous cell carcinomas, 7 (100%) and 5 (71.4%) small cell

carcinomas, and 2 (50.0%) and 3 (75.0%) adenosquamous carcinomas, respectively. While half of 14 adenocarcinomas with high telomerase activity showed neither *TP53* nor *RB1* aberrations, all remaining 13 tumors with high telomerase activity in other histology showed *TP53* and/or *RB1* aberrations (Fig. 1).

***EGFR* and *KRAS* aberrations in lung adenocarcinomas and adenosquamous carcinomas.** Since only in adenocarcinoma, half of tumors with high telomerase activity showed neither *TP53* nor *RB1* aberrations, we surveyed *EGFR* and *KRAS* mutations, known as adenocarcinoma characteristic aberrations in lung cancer, in all adenocarcinoma and adenosquamous carcinoma cases, to explore the genes involved in telomerase activation in *TP53*/*RB1* intact tumors (Table I).

EGFR mutations were detected in 8 of 42 (19.0%) adenocarcinomas and none of 4 adenosquamous carcinomas. All 6 mutations in exon 19 were 15-bp in-frame deletions of the kinase domain, 4 were delE746-A750 (3 showed 2235-2249 deletion: 1 = translation initiation site, 1 showed 2236-2250 deletion) and two were delL747-T751 (2239-2253 deletion). In exon 20, one in-frame insertion of 12 nucleotides was found as insQANP773-776 (2317-2328 insertion CAGGCGAA CCCC). One tumor had a T/G missense mutation L858R in exon 21. Most of these mutations have previously been reported, with the exception of the in-frame insertion in exon 20. There was no tumor that had bi-allelic *EGFR* mutations.

EGFR amplification was evaluated by quantitative real-time PCR in all adenocarcinoma and adenosquamous carcinoma tissues with 27 corresponding adjacent normal lung tissues as control. Since the differences in threshold cycle (Ct) between control gene *TFRC* and *EGFR* were < 0.8 in all adjacent normal lung tissues examined (-0.531-0.796; median ≤ 0.3), we considered the tumors with a Ct difference > 0.8 as having *EGFR* amplification (Fig. 2, cases with asterisk). According to this criteria, 5 of 42 adenocarcinomas (11.9%), including 3 with *EGFR* mutations, but no adenosquamous carcinomas, were determined as having *EGFR* amplification, and this result was confirmed by using another control gene *COG5*, which locates in the opposite arm of the same chromosome 7, except for case 38, indicating the feasibility of this cut-off value.

In 18 (42.9%) of 42 lung adenocarcinomas, a known silent single nucleotide polymorphism was detected at nucleotide 2361 (G/A; Q787Q) in exon 20 (17 heterozygotes and one homozygote; allele frequency, 22.6%) and 1 heterozygote (25%) in 4 adenosquamous carcinomas. The allelic balance observed in heterozygotes was compatible with the amplification data obtained by real-time PCR.

The allelic distribution of *EGFR* intron 1 repeat polymorphism in adenocarcinoma patients was determined by fragment analysis (Fig. 3). The incidence of repeat number was (CA)₁₅=6 (7.1%), (CA)₁₆=10 (11.9%), (CA)₁₇=3 (3.6%), (CA)₁₈=1 (1.2%), (CA)₁₉=0, (CA)₂₀=59 (70.2%), (CA)₂₁=1 (1.2%), (CA)₂₂=3 (3.6%) and (CA)₂₃=1 (1.2%), while that in adenosquamous carcinoma was (CA)₁₆=1 and (CA)₂₀=7. Data of allelic balance was compatible with the *EGFR* amplification classification obtained by real-time PCR.

KRAS codon 12 mutation was found in 2 adenocarcinomas and a codon 13 mutation was found in 1 adenosquamous carcinoma (Table I).

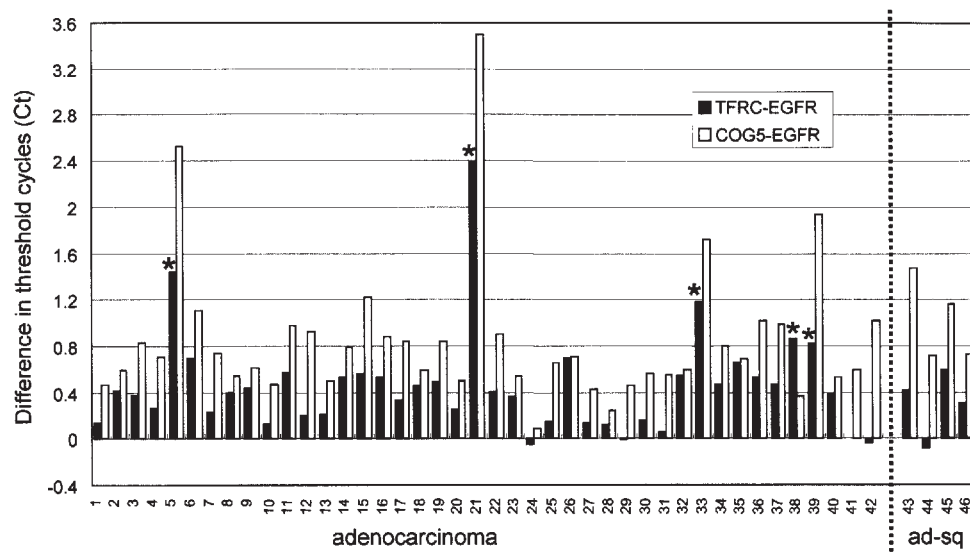


Figure 2. Detection of *EGFR* amplification by TaqMan quantitative real-time PCR system. The vertical value is the difference in threshold cycles (Ct) between *EGFR* and different chromosome control gene *TFRC* (closed bar) or different arm of the same chromosome 7 control gene *COG5* (open bar). *EGFR* amplification was defined when the Ct (*TFRC*)-Ct (*EGFR*) was ≥ 0.8 (5 cases with asterisk). This cut-off value was based on the differences in the adjacent non-cancerous lung tissues.

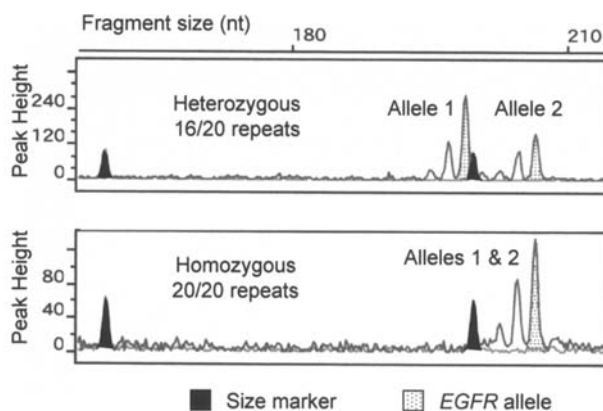


Figure 3. Genotyping of *EGFR* intron 1 polymorphism by fragment analysis. (CA)_n repeat number was determined by the fragment size. Allelic imbalance was defined when the peak height ratio of heterozygous bands was $<50\%$ or $>200\%$ of expected ratio calculated from the normal cells.

Table II. Relationship between genetic aberrations in 42 adenocarcinomas of the lung (p-values calculated by Fisher's exact test).

Aberration no.	<i>TP53</i> 15	<i>RB1</i> 11	1p34del 14	<i>EGFR</i> 10
<i>TP53</i>	-	0.0004	0.0148	>0.9999
<i>RB1</i>	-	-	0.0025	0.4103
1p34del	-	-	-	0.2586

Relationship between EGFR activating aberrations (mutation and/or amplification) and other genetic aberrations in adenocarcinoma. In overall, *EGFR* mutations and/or amplification

were found in 10 adenocarcinomas, but not in 4 adenocarcinomas. As shown in Table II, whereas the aberrations in *TP53*, *RB1* and 1p34-pter were mutually related, *EGFR* aberrations showed no significant relationship to either of them (Table II). Although *KRAS* mutation frequency (2 cases) was too small for statistical analysis, it occurred in tumors with *TP53* but not *EGFR* aberrations. Furthermore, while neither *KRAS* mutation nor 1p34-pter deletion was found in the distinct adenocarcinoma subset with high telomerase activity without *TP53* or *RB1* LOH, *EGFR* in-frame deletion with amplification was observed in one case of this subset. Also *EGFR* polymorphisms in intron 1 and exon 20 showed no relationship with *EGFR* activating aberrations or other genetic events.

Discussion

In lung cancer, one third of non-small cell lung cancer (NSCLC) and most small cell lung cancer (SCLC) tissues have strong telomerase activity similar to that of immortal cancer cell lines (3). One model of telomerase activation in cancer cells is that many serial and clonal selections accumulating multiple genetic aberrations promote additional cell divisions and require activation of telomerase to continue cell division. In fact, SCLC shows abnormalities in chromosomes 3p, 13q (*RB1* locus), and 17p (*TP53* locus) more frequently than NSCLCs (1). In the present study, we demonstrated that there are two subsets in lung adenocarcinomas with high telomerase activity: some adenocarcinomas with high telomerase activity had both *TP53* and *RB1* aberrations as observed in most squamous cell carcinomas or SCLCs, while the remaining half of adenocarcinomas with high telomerase activity had neither (Fig. 1). For the latter subset, no possible factors that might promote telomerase activation have been found. Since *EGFR* activating aberrations (mutations and/or amplification) are primarily found in a distinct population with adenocarcinoma of the lung (9,18,19,28,29), we investigated the

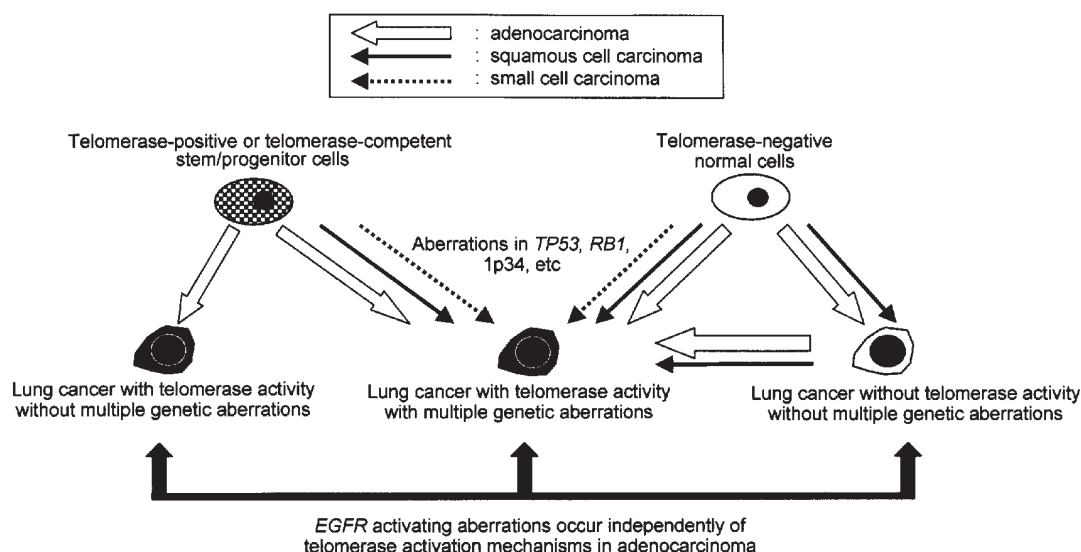


Figure 4. Our hypothesis on the telomerase activation mechanism in lung cancer. Only in adenocarcinoma is there a distinct subset with high telomerase activity without any common genetic aberrations, that may have arisen from a telomerase-positive or telomerase-competent cell, like a stem cell that has low/nil telomerase activity but can upregulate it upon proliferation. Meanwhile, *EGFR* activating aberrations occur in any subset of lung adenocarcinoma independently of other genetic aberrations or telomerase activation mechanisms.

relationship between *EGFR* activating aberrations and adenocarcinoma subgroups classified by telomerase activation mechanisms. Then we found that one case had an in-frame deletion in *EGFR* among the 7 adenocarcinoma cases with high telomerase activity without *TP53/RB1* LOH, while the remaining 6 cases in this subset still showed no genetic aberrations. Furthermore, whereas the aberrations in *TP53*, *RB1* and 1p34-pter were mutually related in all adenocarcinomas examined, *EGFR* aberrations showed no relationship to either of them (Table II). These findings indicate that *EGFR* activating aberrations occur independently with other genetic aberrations or telomerase activation in carcinogenesis of lung adenocarcinoma, and the possibility that the distinct subset of lung adenocarcinoma with high telomerase activity without any common genetic aberrations may have arisen from a telomerase-positive or telomerase-competent cell, like a stem cell (30) that has low/nil telomerase activity but can upregulate it upon proliferation (Fig. 4).

In conclusion, we demonstrated that activation of *EGFR* pathway, *EGFR* mutation and/or amplification, may be involved in both carcinogenesis mechanisms of lung adenocarcinoma in which telomerase is activated through *TP53/RB1* aberrations or without them. There is no relationship between *EGFR* mutation and/or amplification and other genetic aberrations common in lung cancer. Elucidation of the telomerase activation mechanism in the characteristic subset of adenocarcinoma with high telomerase activity without any genetic aberrations might shed light on cancer stem cell study in solid tumors.

Acknowledgements

For providing sample tissues, we are very grateful to Professor K. Inai at the Department of Pathology and Professor N. Kohno at the Department of Molecular and Internal Medicine, Hiroshima University and Drs N. Senoo (Hiroshima City

Hospital), M. Nishiki, Y. Matsuura and T. Toge (Hiroshima University at the time of sample preparation). We also thank Ms. I. Fukuba, Ms. K. Nukata and Ms. M. Wada in our departments, for their technical support. Part of this study was carried out at the Department of Molecular and Internal Medicine and the Analysis Center of Life Science, Hiroshima University. This study was partly supported by Grants-in Aid for Scientific Research from the Ministry of Education, Culture, Science, Sports and Technology, and the Ministry of Health, Labour and Welfare of Japan.

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