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A Study of Efflux Pump Genes in *Mycobacterium tuberculosis* Clinical Isolates

Sun Young Cho[1], You Sun Nam[2], Hyung-Seok Yang[1], Yun Soo Soh[3], Min Jin Kim[4], Hee Joo Lee[1]

[1]Department of Laboratory Medicine, School of Medicine, Kyung Hee University, [2]Department of Biomedical Science, [3]Department of Medicine, Kyung Hee University Graduate School, [4]Seegene Medical Foundation, Seoul, Korea

The efflux pump system has been suggested as an important mechanism in the drug resistance of *Mycobacterium tuberculosis* (MTB). In this study, molecular analysis of five genes in the efflux pump system of MTB isolates from Korean patients was performed in order to identify appropriate molecular targets. In this study, 35 culture-positive specimens were included. PCR was performed for five efflux genes, *mmpL7*, *efpA*, *mmr*, *p55* and *tap*-like gene. In the 35 clinical isolates, molecular analysis of five kinds of efflux pump genes was performed. Only one clinical isolate showed negative PCR results for all five efflux pump genes. All the rest 34 isolates presented concurrent positive results for the five efflux pump genes. In the near future, gene expression study with quantitative PCR should be performed using these genes. **(Ann Clin Microbiol 2014;17:65-68)**

Key Words: Multidrug efflux pump genes, *Mycobacterium tuberculosis*, Drug resistance

It has been reported that decreased drug permeability contributes to phenotypic drug resistance of *Mycobacterium tuberculosis* (MTB) [1,2]. The efflux pump system has been suggested as one of other mechanisms involving drug resistance in MTB [3-6]. A high prevalence of mycobacterial infection to make a special concern in the public health problem in Korea was reported [7]. Nevertheless, there is no epidemiologic study for efflux pump genes in Korean clinical isolate of MTB. In this study, we plan to investigate efflux pump genes of clinical isolates of MTB from Korean patients and to suggest the appropriate target selection for the study about efflux pump genes of MTB.

This study was conducted at Kyung Hee University Hospital, Seoul, Korea, from October 2011 to June 2012. During these periods, 35 specimens showing culture positive results in solid media, and stored in incubator were finally enrolled in this study.

The primer sequences for 5 efflux genes, *mmpL7*, *efpA*, *mmr*, *p55* and *tap*-like gene, have previously been described by Rodrigues et al. and DNA preparation for PCR reaction of efflux genes was done according to our previous study (Table 1) [8,9]. Final 2 μL of template DNA was taken for the PCR reaction with *Maxime* PCR PreMix (*i*-Taq) (*iNtRON* Biotechnology, Sungnam, Korea). PCR was done in LifePro Thermal Cycler (Bioer Technology Co., Ltd, Hangzhou, China). Thermal cycling conditions were as follows: initial denaturation step at 95°C for 5 min, 40 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min; and a final extension step at 72°C for 5 min.

Molecular analysis of 5 kinds of efflux pump genes was performed in total 35 clinical isolates. Laboratory characteristics of these isolates were presented in Table 2. Only one clinical isolate (specimen serial number 33) showed negative results in all of 5 kinds of efflux pump gene PCR (Fig. 1). All the rest 34 isolates presented concurrent positive results in 5 efflux pump genes.

In this study, with molecular analysis of 5 genes in efflux pump system, we investigated efflux pump genes of MTB isolates from Korean patients, to choose and suggest appropriate molecular targets. Almost all isolates (34/35), only except one

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Correspondence: Hee Joo Lee, Department of Laboratory Medicine, School of Medicine, Kyung Hee University, 1 Hoegi-dong, Dongdaemoon-gu, Seoul 130-702, Korea. (Tel) 82-2-958-8672, (Fax) 82-2-958-8609, (E-mail) leehejo@khmc.or.kr

Table 1. Primers for molecular analysis in 5 efflux pump genes

	Target gene	Primer sequence (5'-3')	Size of PCR product	Transporter family	Drug	References
A	*Rv2942* (*mmpL7*)	F) TACCCAAGCTGGAAACAA R) CCGTCAGAATAGAGGAACAG	214 bp	RND	INH	[3], [9]
B	*Rv2846c* (*efpA*)	F) ATGGTAATGCCTGACATCC R) CTACGGGAAACCAACAAAG	131 bp	MFS	INH, ETH	[3], [9]
C	*Rv3065* (*mmr*)	F) AACCAGCCTGCTCAAAAG R) CAACCACCTTCATCACAGA	221 bp	SMR	INH	[9], [10]
D	*Rv1410c* (*p55*)	F) AGTGGGAAATAAGCCAGTAA R) TGGTTGATGTCGAGCTGT	198 bp	MFS	STR, RIF, INH	[3], [9]
E	*Rv1258c* (*tap*-like gene)	F) AGTTATAGATCGGCTGGATG R) GTGCTGTTCCCGAAATAC	268 bp	MFS	STR, RIF, OFX, INH	[3], [9]

Abbreviations: F, forward; R, reverse; bp, base pairs; RND, resistance nodulation division; MFS, major facilitator super-family; SMR, small multidrug resistance family; INH, isoniazid; RIF, rifampicin; OFX, ofloxacin.

Table 2. Characteristics of 35 clinical isolates in this study

No.	Specimen	Sex/Age	AFB smear	Culture (L)	Culture (S)	Drug susceptibility test*
1	Sputum	M/37	1+	Negative	1+	Susceptible for all agents tested
2	Sputum	M/27	1+	MTB	1+	Susceptible for all agents tested
3	Urine, clean	F/69	Negative	MTB	2+	Susceptible for all agents tested
4	Sputum	M/26	Negative	Negative	1+	Susceptible for all agents tested
5	Urine, clean	F/73	Negative	NA	1+	NA
6	Sputum	M/43	2+	Negative	2+	Susceptible for all agents tested
7	Sputum	M/52	Negative	NA	1+	Susceptible for all agents tested
8	Sputum	F/25	3+	NA	2+	Susceptible for all agents tested
9	Pleural mass	F/81	Negative	MTB	1+	Susceptible for all agents tested
10	Pleural fluid	F/46	Negative	MTB	1+	Susceptible for all agents tested
11	Sputum	F/46	1+	MTB	2+	Susceptible for all agents tested
12	Sputum	M/15	Negative	MTB	1+	Susceptible for all agents tested
13	Sputum	F/25	Negative	MTB	1+	NA
14	Sputum	F/79	Negative	MTB	1+	NA
15	Sputum	F/80	Negative	Negative	1+	NA
16	Sputum	F/79	Negative	MTB	1+	Susceptible for all agents tested
17	Sputum	M/71	1+	MTB	1+	Susceptible for all agents tested
18	Sputum	F/72	Negative	MTB	1+	Susceptible for all agents tested
19	Sputum	M/32	Negative	MTB	1+	Susceptible for all agents tested
20	Sputum	F/54	Negative	MTB	2+	Susceptible for all agents tested
21	Bronchial washing	F/54	Negative	MTB	1+	Susceptible for all agents tested
22	Sputum	M/81	2+	MTB	1+	Resistant for INH, RIF, and EMB
23	Sputum	M/51	1+	Negative	2+	Susceptible for all agents tested
24	Bronchial washing	F/69	Negative	MTB	1+	Susceptible for all agents tested
25	Sputum	M/64	Negative	MTB	1+	Susceptible for all agents tested
26	Sputum	M/54	1+	MTB	1+	Susceptible for all agents tested
27	Mediastinum	F/37	Negative	MTB	1+	NA
28	Bronchial washing	M/17	Negative	MTB	1+	Resistant for SM, RIF, and RFB
29	Bronchial washing	M/39	Negative	MTB	1+	Susceptible for all agents tested
30	Sputum	M/72	Negative	Negative	1+	Susceptible for all agents tested
31	Sputum	F/72	Negative	MTB	1+	Susceptible for all agents tested
32	Sputum	F/80	Negative	MTB	1+	Susceptible for all agents tested
33	Sputum	F/81	Negative	MTB	1+	Resistant for INH
34	Sputum	F/81	1+	MTB	1+	Resistant for INH
35	Sputum	F/27	1+	NA	1+	Susceptible for all agents tested

Abbreviations: L, liquid; S, solid; F, female; M, male; NA, non-available; MTB, *Mycobacterium tuberculosis*; INH, isoniazid; RIF, rifampicin; EMB, ethambutol; SM, streptomycin; RFB, rifabutin.

*Tested anti-TB agents for the drug susceptibility test include amikacin, capreomycin, cycloserin, ethambutol, isoniazid, kanamycin, levofloxacin, moxifloxacin, ofloxacin, P-aminosalicylic acid, prothionamide, pyrazinamide, rifampicin and streptomycin.

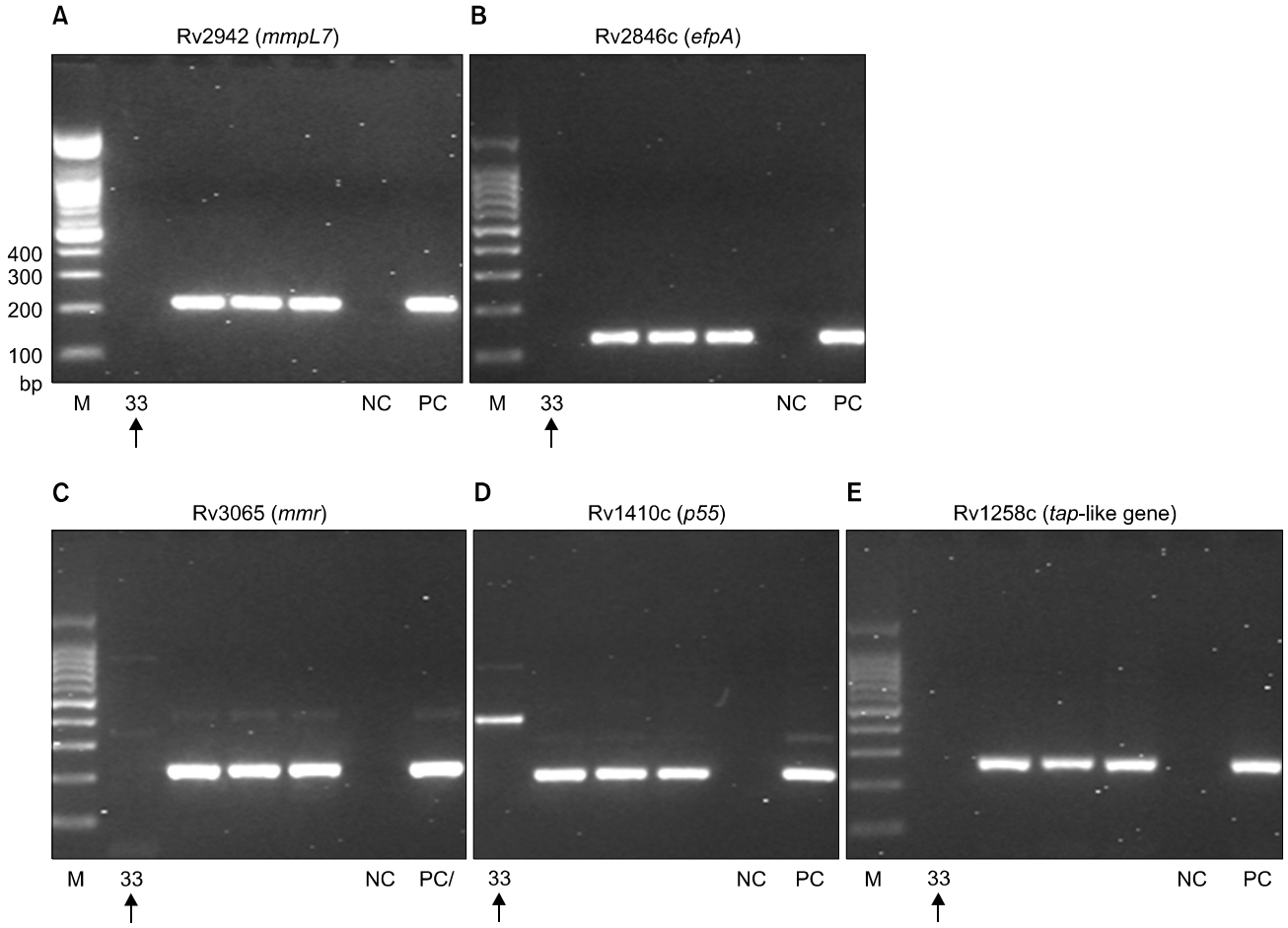

Fig. 1. Molecular analysis of efflux pump genes in clinical specimens of Korean patients. In total 35 specimens showing the positivity for *M. tuberculosis*, only one clinical strain shows negative results in all of 5 kinds of efflux pump gene PCR (specimen serial number 33, arrows). The rest of strains present positive results in 5 efflux pump genes, simultaneously. M, size marker; NC, negative control; PC, positive control.

case, presented positive results in PCR tests of 5 kinds of efflux pump genes. Because the efflux pump induction is an important step in the evolution of mycobacterial drug resistance, gene expression study should be followed in these genes, with a large number of clinical isolates [6]. Comparisons of gene expression levels according to treatment courses and drug exposure can also provide information to understand the role of efflux pump genes of MTB.

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=국문초록=

임상 분리된 결핵균에서의 Efflux Pump Gene에 대한 선행연구

[1]경희대학교 의과대학 진단검사의학교실, 경희대학교 대학원 [2]기초의과학과, [3]의학과, [4]씨젠의료재단

조선영[1], 남유선[2], 양형석[1], 소윤수[3], 김민진[4], 이희주[1]

Efflux pump system은 약제 내성 결핵균의 중요한 기전 중 하나로 제안되고 있다. 이 연구는 efflux pump system의 5가지 유전자에 대한 분석을 통해, 한국 환자들로부터 분리된 결핵균의 적절한 표적을 선택하고 제안하기 위해 시행되었다. 이 연구에서는 양성 동정결과를 보이는 35 검체가 분석되었다. *mmpL7*, *efpA*, *mmr*, *p55*, 그리고 *tap*-like gene 등 5종류의 efflux gene에 대해 PCR을 시행하여 35개의 분리된 결핵 균주를 분자적 방법으로 분석하였다. 그 결과 오직 하나의 분리 균주에서 5종류의 efflux pump gene PCR에 대해 음성 결과를 보였다. 나머지 34개의 분리 균주에서는 5종류의 efflux pump gene에 대해 동시 양성 결과가 나왔다. 앞으로 이 유전자들에 대한 PCR 정량 검사를 통해 유전자 발현 조사가 진행되어야 할 것이다. [Ann Clin Microbiol 2014;17:65-68]

교신저자 : 이희주, 130-702, 서울시 동대문구 회기동 1번지
경희의료원 진단검사의학과
Tel: 02-958-8672, Fax: 02-958-8609
E-mail: leehejo@khmc.or.kr