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SHORT COMMUNICATION

hMSH2 and hMLH1 gene expression patterns differ between lung adenocarcinoma and squamous cell carcinoma: correlation with patient survival and response to adjuvant chemotherapy treatment

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ABSTRACT

Background: **We recently showed that the mRNA levels of mismatch repair (MMR) proteins in non-small cell lung carcinoma (NSCLC) tissue specimens and the phenotypic translation of molecular MMR data refines the biology of the MMR system with consequent diagnostic implications in the clinical assessment of lung cancer patients.**

Methods: **hMLH1 and hMSH2 mRNA expression was previously evaluated by qPCR for 29 NSCLC patients (13 with squamous cell carcinoma [SQC] and 16 with adenocarcinoma [ADC]) and MMR mRNA levels were converted into clinically distinct phenotypic entities. In this study, we evaluated the correlation of the hMSH2 and hMLH1 mRNA phenotypes with patient survival and their response to adjuvant chemotherapy.**

Results: **hMSH2 and hMLH1 mRNA phenotypic distribution differed between SQC and ADC. The MMR phenotypes differed also between advanced and early stage SQC. SQC patients with an increased hMSH2 expression had a better outcome than patients with a reduced hMSH2 expression. However, ADC patients with an increased hMSH2 expression had a poor outcome compared to those with low hMSH2 levels. SQC patients with a high hMSH2 expression exhibited a better response to adjuvant chemotherapy, whereas ADC patients with high hMSH2 levels had a poor response. ADC patients with low hMSH2 levels showed good response to adjuvant chemotherapy compared to SQC patients bearing the same phenotypic profile.**

Conclusions: **Our findings show that MMR mRNA phenotypes may be added to the known biological differences between SQC and ADC. hMLH1 and hMSH2 phenotypes distributed differently according to the NSCLC stage. Distinct MMR mRNA phenotypes in SQC and ADC corresponded to patient response to adjuvant chemotherapy.**

Key words: MMR, hMSH2, hMLH1, NSCLC, Survival, Chemotherapy

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INTRODUCTION

Defects in DNA mismatch repair (MMR) proteins account for the predisposition and onset of an hereditary form of colorectal cancer, HNPCC, due to mutations in the coding regions of MMR genes that lead to non-functional proteins (1, 2). In sporadic cancers, genetic alterations in the regulatory regions of the MMR genes caused by hypermethylation contribute to carcinogenesis (3, 4). However, increased levels of MMR gene expression observed in some sporadic cancers correlated with tumor aggressiveness (5-9). A clear balance in MMR protein levels therefore needs to be maintained to avoid cancer predisposition and onset.

We recently showed that the MMR mRNA levels in non small-cell lung carcinoma (NSCLC) correlate with the clinical condition of the patients, and we suggested that the phenotypic translation of molecular MMR data refines the biology of the MMR system with consequent diagnostic implications in the clinical assessment of lung cancer patients (9). Yet, the distribution of mRNA phenotypes in NSCLC, and their correlation with patient outcome following post-operative chemotherapy (POC) treatment is of major significance.

In the present study, we investigated the distribution of

the phenotypes, R2R1, R2r1, r2R1 and r2r1, in squamous cell carcinoma (SQC) and adenocarcinoma (ADC) of the lung, and examined their correlation with patient survival and response to adjuvant chemotherapy treatment.

MATERIALS AND METHODS

Tissue collection and molecular analysis

Freshly frozen tissue samples were collected from 29 patients with NSCLC, who underwent surgery and were treated at the Medical Center of Athens, Greece, as described in a previous study (9).

Our cohort comprised 13 squamous cell carcinomas (SQC) and 16 adenocarcinomas (ADC) of the lung. Quantitative real-time PCR analysis was previously used to obtain the mRNA levels of hMSH2 and hMLH1 relative to the reference gene hPBGD (9). The MMR mRNA levels were then converted into clinically distinct phenotypic entities by these working criteria, based on the hypothesis that reduced mRNA levels result in less or non-functional MMR. We identified 4 distinct combinations of MMR mRNA phenotypes: R2R1, R2r1, r2R1 and r2r1. Specifically, r2 and r1 corresponded to the mRNA ratios of hMSH2/hPBGD and hMLH1/hPBGD ≤1; R2 and R1 corresponded to the mRNA ratios of hMSH2/hPBGD and hMLH1/hPBGD ≥1. The clinicopathological data of our cohort as well as the mRNA phenotypic patterns of all patients are shown in Table I.

Statistical analysis

Statistical analysis of the results was performed with the SPSS 14.0 software for Windows. The MMR

TABLE I - CLINICOPATHOLOGICAL FEATURES OF LUNG CANCER PATIENTS, CHEMOTHERAPY, mRNA MMR PHENOTYPES AND SURVIVAL DATA

Sample n	Patient age (years)	Gendera (M/F)	Tumor typeb	Histological tumor stagec	Chemd	mRNA MMR phenotype	Survivale
1	42	M	AD	III	+	R2r1	D (6 m)
2	68	M	AD	II	-	R2R1	F
3	69	M	AD	I	+	R2R1	D (1 y)
4	57	M	AD	I	-	r2R1	F
5	61	M	AD	III	+	R2R1	D (3.5 y)-R
6	75	M	AD	III	+	R2R1	D (3.25 y)-M
7	66	M	AD	III	+	r2r1	F-R
8	71	M	AD	III	+	r2R1	D (20 m)
9	47	M	AD	III	+	r2r1	F
10	60	M	AD	Iv	+	R2R1	D (2.5 y)-R
11	61	F	AD	III	-	R2r1	F
12	72	F	AD	III	+	R2R1	D (2 y)-R
13	61	F	AD	II	-	R2r1	F
14	82	F	AD	II	-	r2R1	F
15	54	F	AD	III	+	r2R1	F
16	77	F	AD	I	-	r2r1	F
17	72	M	SQ	III	-	r2R1	D (5 d)
18	69	M	SQ	I	-	R2r1	F
19	58	M	SQ	III	+	r2r1	D (1 y)
20	73	M	SQ	II	-	R2R1	F
21	46	M	SQ	I	-	R2R1	F
22	56	M	SQ	II	+	R2R1	F
23	58	M	SQ	I	-	r2R1	F
24	63	M	SQ	III	+	r2R1	F
25	60	M	SQ	II	+	R2R1	F
26	71	M	SQ	II	+	r2R1	D (1 y)
27	53	F	SQ	I	+	R2R1	F
28	61	F	SQ	I	+	R2r1	F
29	65	F	SQ	II	+	R2r1	F

[a]Gender: M, male; F, female
[b]Tumor type: ADC, adenocarcinoma; SQC, squamous-cell carcinoma
[c]Histological tumor stage according to WHO
[d]Chemotherapy: +, with chemotherapy treatment; -, without chemotherapy treatment
[e]Survival: F, free of disease; D, dead; R, recurrence of disease; M, metastasis; d, days; m, months; y, years

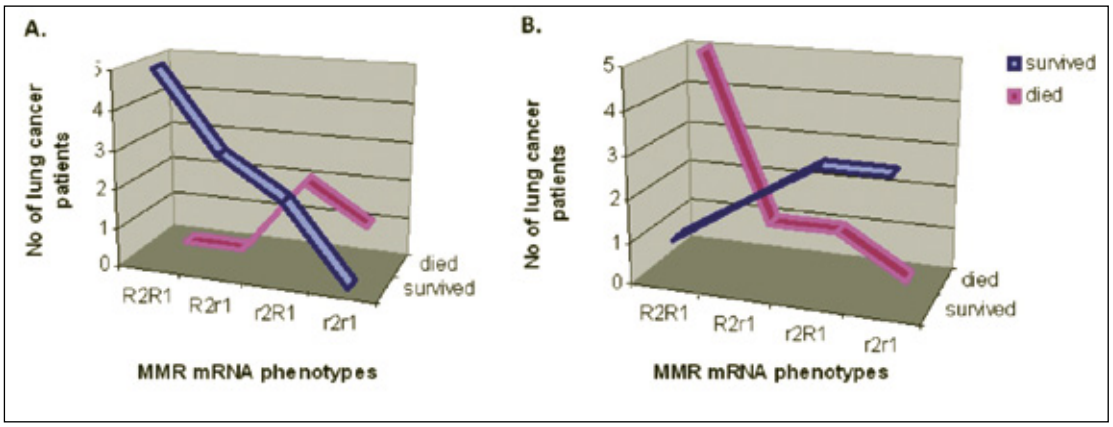

Fig. 1 - *Distribution of SQC (A) and ADC (B) NSCLC patients relative to their outcome and MMR mRNA phenotyping.*

gene expression levels were initially evaluated by the one-sample Kolmogorov-Smirnov goodness-of-fit test to determine whether they adhered to a normal distribution pattern. A chi-square (χ^2) test was performed to examine the distribution of the 4 MMR mRNA phenotypes in the tumor tissue specimens. Pearson's correlation and the χ^2 test were used to correlate patient survival with chemotherapy treatment, as well as to compare the phenotypic distribution relative to the patient outcome after chemotherapeutic treatment. The Kaplan-Meier curves were used to estimate survival as a function of time, and survival differences were assessed by the log-rank test.

RESULTS

Distribution of MMR phenotypes in SQC and ADC

The hMSH2 and hMLH1 phenotypes exhibited different distributions between SQC and ADC. The hMSH2 levels were reduced (phenotypes r2R1 and r2r1) in ADC of early histopathological stages, compared with SQC of higher stages ($p<0.001$).

MMR genes or the dominant hMSH2 were normally or highly expressed (phenotypes R2R1 and R2r1) in early SQCs compared with late histopathological stages (phenotypes r2R1 and r2r1) ($p<0.05$). By contrast, ADCs did not exhibit a similar trend of reduction (r2R1 and r2r1) in advanced histopathological stages. Moreover, an increased MMR expression occurred more frequently in advanced-stage tumors than early-stage tumors. Thus, a clear difference in MMR phenotypic distribution between the advanced stages of SQC and ADC was observed ($p<0.001$) (Tab. II).

Patient survival and MMR phenotypes

Twenty-four out of 29 patients (83%) were disease free 18 months after surgery, 5 patients (17%) died earlier, while 4 (14%) experienced disease recurrence or metastasis. Seventeen out of 29 (59%) patients were disease free 3.5 years after surgery, while 2 of the patients that survived showed disease recurrence or metastasis (Tab. I). The median follow-up time for the ADC and SQC patients was, respectively, of 50 months and 52 months. The Kaplan-Meier curve results did not reveal any significant difference in overall patient survival among R2R1, R2r1, r2R1 and r2r1 MMR phenotypes.

Among the SQCs cases, 80% of the patients who survived exhibited the mRNA phenotypic combination R2R1 or R2r1, while the 3 deceased patients exhibited the mRNA phenotypic combination r2R1 or r2r1. In ADCs, an almost inverse phenotypic distribution was noted ($p<0.001$, χ^2 test). The majority of the patients who survived for more than 3.5 years (67%) expressed r2R1 or r2r1, whereas 85.7% of the patients with phenotypes R2R1 or R2r1 did not survive (Tab. I and Fig. 1).

Eighteen patients received adjuvant chemotherapy with cisplatin. Of these, 9 patients survived 3.5 years after surgery and 8 were disease free, indicating a positive correlation between chemotherapy and survival (Pearson's r=0.860).

We observed a different distribution of the MMR mRNA phenotypic profiles between SQC and ADC patients who survived and received POC. Eight lung SQC patients received POC, of whom 6 were disease free 3.5 years after surgery. Five of these 8 patients exhibited

TABLE II - DISTRIBUTION OF MMR mRNA PHENOTYPES IN SQC AND ADC, RELATIVE TO THEIR HISTOPATHOLOGICAL STAGE

MMR mRNA phenotype	SQC		ADC	
	Histopathological stage			
	I-II	III	I-II	III-IV
R2R1	5	-	2	4
R2r1	3	-	1	2
r2R1	2	2	2	2
r2r1	-	1	1	2

the R2R1 or R2r1 phenotypes. The remaining 2 POC patients who did not survive had the r2R1 or r2r1 MMR phenotypes ($p<0.01$) (Tabs. I and III). Eleven lung ADC patients received POC. Two of these patients were disease free for 3.5 years after surgery and exhibited the r2R1 or r2r1 MMR mRNA phenotypes. However, the remaining 9 patients did not survive for more than 3.5 years, and most of them (7 patients, 78%) exhibited the R2R1 or R2r1 MMR phenotypes ($p<0.001$).

SQC patients who responded to POC had early-stage tumors and exhibited an elevated hMSH2 expression (R2R1 or R2r1 MMR mRNA phenotypes), whereas those who did not respond to POC had late-stage tumors and a reduced hMSH2 expression (r2R1 or r2r1 MMR mRNA phenotypes). ADC patients who responded to POC had late-stage tumors, and exhibited a reduced hMSH2 expression (r2R1 or r2r1 MMR mRNA phenotypes), whereas patients who did not respond to POC had early-stage tumors and exhibited elevated hMSH2 expression levels (R2R1 or R2r1 MMR mRNA phenotypes) (Tab. I).

DISCUSSION

Differences in the biological behavior of SQC and ADC of the lung are well known (10, 11). Our findings show that MMR mRNA phenotypes may be added to the known biological differences of these 2 subtypes. Primary lung SQCs showed a high expression of the MMR transcripts, as opposed to ADCs, which exhibited a reduced MMR gene expression. Our findings are in agreement with those of Cooper et al who showed normal MMR expression in all cases of squamous metaplasias and squamous dysplasias (both lesions are precursors of SQC) (12). We also support the findings of Cooper et al, who showed a reduced MMR expression in non-invasive ADC (12). In the present study, we showed that MMR phenotypes differ between the distinct pathological stages of NSCLC. During the early histological stages of lung SQCs, the elevated DNA repair expression levels protect the cells from enhanced tumorigenesis. In advanced stages, the repair system becomes deficient, leading to a more aggressive behavior. By contrast, we observed that the early-stage lung ADCs had reduced levels of hMSH2, a crucial component of the DNA MMR system. Such a primary defect in DNA repair would enhance genetic instability and contribute to accelerated tumorigenesis. On the other hand, the large proportion of R2R1 and R2r1 MMR phenotypes in the late stages of lung ADC compared with their SQC counterparts would support their different pathogenesis and may correlate with a more severely affected DNA MMR mechanism. Thus, a decreased reparative function correlates with MSH2 domain-specific mutations. These mutations are considered to induce functional destabilization or abrogation of protein-DNA interactions (13-15). Notably, immunohistochemistry may not be the best method for detecting certain MMR defects, since the R2R1 phenotypes may represent non-functional mutated proteins.

In general, prediction of tumor response to POC has been problematic (16). To the R2R1 phenotype corresponds an increased expression level of hMSH2 and hMLH1. hMSH2 is a crucial component of the DNA MMR system, as it recognizes mismatches and is involved in promoting apoptosis in the presence of certain types of DNA damage, e.g. after chemotherapeutic agents (17, 18). This dual role of the protein may explain the proportion of patients presenting an R2R1 phenotype that exhibited a trend for improved survival following chemotherapy, compared to the r2r1 phenotype in SQCs. Additionally, SQC patients with an r2r1 phenotype (homozygous reduced) showed no benefit from chemotherapy treatment, possibly due to the fact that apoptotic pathways were partially abrogated. We found that the heterozygous reduced phenotype (r2R1) of the same lung tumors was similar to the homozygous reduced phenotype (r2r1), suggesting that the reduced mRNA levels of one MMR gene are sufficient to hinder apoptosis, possibly due to a mutated MSH2 protein. Thus, both the heterozygous (R2r1) and homozygous (R2R1) phenotype in SQC appear to be better in combination with POC compared to the r2R1 phenotype.

The observations here described showed that both MMR phenotypes, r2r1 and R2r1, were found to be positive indicators of survival without chemotherapeutic treatment. Earlier reports are in line with our observations, showing that patients carrying MMR-deficient tumors have

TABLE III - DISTRIBUTION OF MMR mRNA PHENOTYPES IN SQC AND ADC RELATIVE TO POST-OPERATIVE CHEMOTHERAPY (POC) TREATMENT RESPONSE

MMR mRNA phenotype	SQC patients			ADC patients		
	POC	survived	died	POC	survived	died
R2R1	3	3	0	5	0	5
R2r1	2	2	0	1	0	1
r2R1	2	1	1	2	1	1
r2r1	1	0	1	2	1	1

a survival advantage over those devoid of comparable deficiencies in the MMR system (19, 20).

In conclusion, our findings show that different MMR phenotypes represented SQC and ADC of NSCLCs. Additionally, benefits of POC were found to correlate with distinct MMR phenotypes in either histological type of NSCLCs. This study should be followed up in a larger cohort of NSCLC patients, with or without POC treatment, as to provide further evidence for our results.

Conflict of interest statement: None.

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