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# NQO1 Polymorphisms and Prognosis of Non-small Cell Lung Cancer after Radiation Therapy

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## 1. Introduction

NQO1 [NAD(P)H:quinone oxidoreductase 1, previously called as DT-diaphorase] is a cytosolic flavoenzyme that catalyzes the two-electron reduction of the more toxic quinone to less toxic hydroquinone [1]. NQO1 was reported to be a key player in β-lapachone induced apoptosis. NQO1 gene is located on chromosome 16q22. NQO1 polymorphisms are manifested as a C→T substitution at nucleotide position 609, and this is resulted as a proline-to-serine change at position 187 in the amino acid sequence of the protein. This NQO1 polymorphism has been reported to lead to greatly diminished levels of NQO1[2]. We hypothesized that NQO1 after radiation therapy increased the apoptosis, and that NQO1 polymorphism, the difference in NQO1 activity, had an effect on the survival outcome of NSCLC patients treated with surgery or radiotherapy (Figure 1). We tried to reveal the usefulness of the NQO1 polymorphism in NSCLC patients as a prognostic marker for determining outcome of anti-cancer treatment.

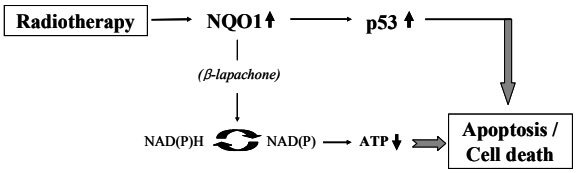


Figure 1. NQO1 and radiotherapy

## 2. Materials and Methods

### *2.1 Study populations and Radiation therapy*

Patients who had been treated with radiation therapy from 2000 to 2003 were recruited at the Asan Medical Center. NSCLC patients (stage I-III) who were histologically proven as squamous cell carcinoma and adenocarcinoma were recruited, and they had a good performance (ECOG 2 or below). Patients with the history of another neoplasm or radiation therapy were excluded. Radiation therapy was performed with curative or postoperative adjuvant aim.

### *2.2 Sampling and Genotyping assay*

Sampled blood was bottled in tubes that contain 1.5ml of anticoagulant. DNA was extracted from the lymphocytes. DNA was amplified by polymerase chain reaction (PCR), and PCR products were then denatured. The primer set for the NQO1 polymorphism was 5´-TCC TCA GAG TGG CAT TCT GC-3´ [3].

### *2.3 Genotyping from tumor tissue: Gel electrophoresis*

Paraffin-embedded tumor tissue was used for DNA extraction form tissue, and it was sliced with 10 *um* thickness. Sliced tumor tissue undergone deparaffinization, lysis, and denaturation. DNA extraction, PCR, and purification were performed. Gel electrophoresis was performed on 3% agarose gel with 0.5X TBE buffer, on charge of 100 voltage during about 30 to 40 minutes (Figure 2).

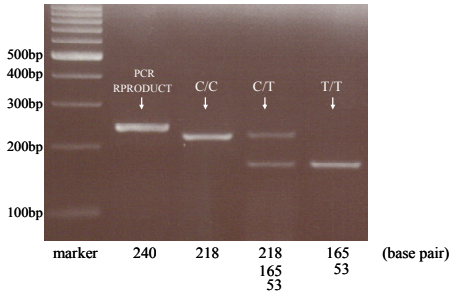


Figure 2. DNA gel elctrophoresis: NQO1 genotypes

## 3. Results

### *3.1 NQO1 polymorphism and patients' characteristics*

NQO1 polymorphism was divided into three groups according to presence of point mutation: 1) C/C, normal

activity, 2) C/T, decreased activity, and 3) T/T, no activity, and the numbers in its type were 62, 99 and 36, respectively. No significant relationship was found between NQO1 polymorphism and the baseline characteristics of the recruited patients

*3.2 Survival outcome according to NQO1 genotype*

We analyzed survival outcome separately by the aim of radiation therapy, curative versus adjuvant. One hundred-five patients belonged to curative group and the other ninety-two patients to adjuvant group.

In curative group, locoregional progression-free survival (LRPFS) was significantly different by NQO1 genotype (p=0.026), but distant metastasis-free survival (DMFS), progression-free survival (PFS), and overall survival (OS) were not different between genotypes (Figure 3).

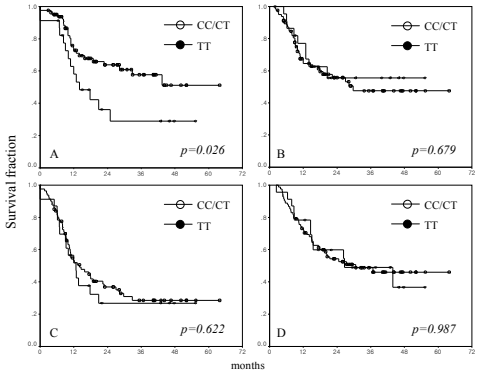


Figure 3. survival outcome by NQO1 genotype (curative radiation therapy)
*A*. LRPFS *B*.DMFS *C*. PFS *D*. OS

In adjuvant group, there was no difference between NQO1 genotype, but LRPFS and PFS had marginal significance by NQO1 activity (p=0.074 and 0.071 by C/C, C/T versus T/T) (Figure 4).

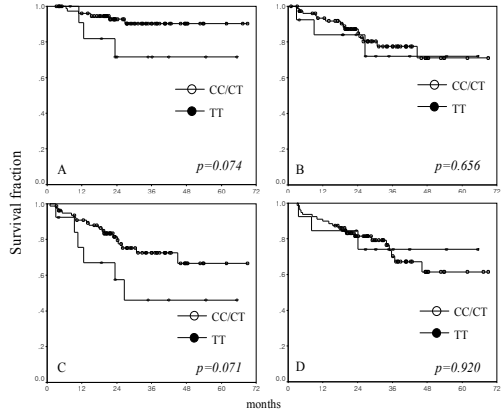


Figure 4. survival outcome by NQO1 genotype (postoperative radiation therapy)
*A*. LRPFS *B*.DMFS *C*. PFS *D*. OS

*3.3 Genotypes from gel electrophoresis versus blood*

DNA gel electrophoresis from tumor tissue was performed for confirming the genotype from blood sample. Until now, electrophoresis was accomplished in only eighteen patients, and the experiment was ongoing. Among nine patients with C/C genotype in blood, seven patients showed C/C genotype, and the other two patients showed C/T genotype. Another nine patients with T/T genotype in blood showed identical genotype in tumor tissue (correlation coefficient=0.949, $p<0.01$). As a result, we assured that the genotype from blood could reflect the genotype directly from tumor tissue, although analyzed patients' number was insufficient.

## 4. Conclusion

We selected the NQO1 gene as an important site of the polymorphism in NSCLC, because NQO1 had a pivotal role in the oxidative status and NQO1 polymorphism lead to the different enzymatic activity according to its genotype. In this study, we analyzed the survival outcome in each group of patients showing the different NQO1 genotype, and suggested the possibility that the NQO1 polymorphism might be used as a prognostic marker in patients receiving radiotherapy in NSCLC. This study showed that locoregional control rate after radiotherapy in NSCLC patients had a significant difference by NQO1 genotype, i.e. NQO1 activity, but this result could not reach to the improvement of overall survival outcome. Progression-free survival (PFS) also could not reach to the statistical significance, but this might be attributed that DMFS greatly affected on the results. Because the surgery and radiotherapy are the locoregional treatment modalities, locoregional outcome can be regarded as more important criterion than overall outcome.

NQO1 polymorphisms could be used as a prognostic marker in non-small cell lung cancer patients who are treated with radiotherapy, although the further study with more patients and long-term follow-up should be promised.

## Acknowledgements

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