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Combination therapy of RRM2 siRNA and endocrine therapy or chemotherapy for treatment of Luminal A breast cancer

February 2016

Graduate School of Pharmacy
Seoul National University
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Combination therapy of RRM2 siRNA and endocrine therapy or chemotherapy for treatment of Luminal A breast cancer

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Submitting a master’s thesis of Public Administration

February 2016

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Confirming the master’s thesis written by Nguyen Thuy Linh
February 2016

Chair
Vice Chair
Examiner
ABSTRACT

Combination therapy of RRM2 siRNA and endocrine therapy or chemotherapy for treatment of Luminal A breast cancer.

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Ribonucleotide reductase M2 (RRM2) subunit is one of two subunits that constitute ribonucleotide reductase, the enzyme that converts ribonucleotide 5'-diphosphates into 2'-deoxyribonucleotides, which are required for DNA synthesis. The overexpression of RRM2 was associated with tumor aggression and higher risk of relapse in even Luminal A— the least aggressive breast cancer subtype. In this study, the therapeutic potential of targeting RRM2 was investigated in Luminal A subtype. Suppression of ribonucleotide reductase M2 subunit synthesis, using small interfering RNA, inhibited cell growth, clonogenicity, wound healing ability and triggered G1 phase cell cycle arrest in Luminal A breast cancer cell lines. Targeting RRM2 as a therapy was demonstrated to show synergistic effect with Tamoxifen and Doxorubicin in the inhibition of MCF-7 cell line. The study suggests that suppression of RRM2 (and its function) is a potential therapeutic strategy and combined therapy of targeting RRM2 and endocrine or chemotherapy is recommended for poor prognosis Luminal A patients whose RRM2 expression is
elevated.

**Keywords**: Luminal A breast cancer; Ribonucleotide reductase M2 subunit; small interfering RNA; Synergistic effect

**Student Number**: 2014-22150
CONTENTS

ABSTRACT ........................................................................................................... i
CONTENTS ......................................................................................................... iii
LIST OF FIGURES .......................................................................................... v
LIST OF TABLES .............................................................................................. vi
INTRODUCTION ............................................................................................... 1
MATERIALS AND METHODS ................................................................. 4
  Patient samples ............................................................................................ 4
  RNA expression of RRM2 in clinical study ................................................ 4
  Cell culture and drug treatment ................................................................. 4
  siRNA and transfection ............................................................................ 5
  Western blot analysis .................................................................................. 5
  Cell proliferation assay ............................................................................. 6
  Wound healing assay .................................................................................. 6
  Cell colony formation assay ..................................................................... 7
  Cell cycle analysis ..................................................................................... 7
  Chou and Tatalay analysis ......................................................................... 8
  Annexin V/ PI (Propidium iodide) staining for apoptosis ....................... 8
  Statistical analysis ..................................................................................... 8
RESULTS ........................................................................................................... 10
  Correlation between RRM2 overexpression and poor outcome in Luminal A
  breast cancer .......................................................................................... 10
  RRM2 is overexpressed in Luminal A breast cancer ................................ 10
LIST OF FIGURES

Figure 1. Expression level of RRM2 at protein level.

Figure 2. RRM2 knockdown reduces proliferation and growth of MCF7 and ZR 75-1 cells.

Figure 3. RRM2 knockdown triggers cell cycle arrest at G0/G1 phase in MCF7 and ZR75-1 cells.

Figure 4. RRM2 knockdown suppresses migration of MCF7 and T47D cells.

Figure 5. Enhanced effect in inhibition of MCF 7 resulting from combination therapy.
LIST OF TABLES

Table 1.  Patient classification into 5 different subgroups based on Hormone status.
Table 2.  Clinical characteristics of breast cancer patients.
Table 3.  Diagnostic significance of RRM2 expression in Breast cancer.
Table 4.  Chou Tatalay drug interaction analysis of RRM2 siRNA and Tamoxifen or Doxorubicin.
INTRODUCTION

Breast cancer is the second most common cancer in the world and is emerging as the most common cancer among women with an estimate of 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers)[1]. For better prognosis and determination of appropriate therapy, breast cancer is classified into luminal A, luminal B, human epidermal growth factor receptor 2 -HER2, and basal-like types on the basis of gene expression profiling [2]. Among them, luminal-A, which is defined as estrogen receptor (ER)-positive and/or progesterone receptor (PR)-positive tumors with negative HER2 and low Ki67, is the most common subtype accounting for 50%-60% of all breast cancers. Although patients with luminal-A breast cancer have best prognosis with significantly lower relapse rate than the other subtypes [3], a small proportion of them experience loco-regional relapse or distant metastasis after 10 years [4]. Therefore, identification of genetic prognostic factors for Luminal A high-risk patient group holds great meaning both to distinguish subgroup of patients who need more intensive adjuvant therapy and to avoid overtreatment to good prognosis subgroup.

The recent discovery of RNA interference (RNAi), a mechanism by which double stranded RNAs mediate sequence-specific gene silencing, provides a new tool in the fight against cancer [5]. RNAi - induced gene silencing hijacks the inhibitory effects of conventional pharmaceuticals, by mainly blocking their targets' function. SiRNA (small interfering RNA) have been extensively used to silence cancer-related gene targets.

In breast cancer treatment, siRNA has been rapidly developing as a promising therapeutic candidate. It is previously reported that anti-RhoA and anti-RhoC siRNAs inhibited the proliferation and invasiveness of MDA-MB-231 breast cancer cells in vitro and in vivo [6]; that delivery of CXCR4-siRNA reduced proliferation and metastasis and induced apoptosis in the HER2(+) breast
cancer [7]; targeted delivery of PLK1-siRNA suppresses Her2+ breast cancer growth and metastasis [8]. These results highlights that knockdown gene expression using siRNA and its potential application is worthy to explore.

RRM2 encodes for the small subunit of the enzyme ribonucleotide reductase, which catalyzes the rate-limiting step of the de novo pathway of DNA generation, reducing the 2′ carbon of a ribonucleoside diphosphate (NDP) to corresponding deoxy-(d)NDP, which is then phosphorylated by nucleoside diphosphate kinase (NDPK) to yield dNTPs. The overexpression of RRM2, followed by alteration in RNR enzyme activity, might supply cancer cells with abundant source of building blocks for DNA synthesis [9], leading to their malignant characteristics. Therefore, targeting RRM2 along with standard treatment therapy for Luminal A holds promising role to eliminate cancer cells.

Ribonucleotide reductase subunit M2 (RRM2) overexpression recently has been correlated with poor outcome of several cancers such as colorectal [10], bladder [11], nasopharyngeal [12], breast cancer and chemo-resistance [13], suggesting its role as a potential prognostic factor. Previously, our lab has identified through genetic analysis of 820 formalin-fixed paraffin embedded tumor samples, we have identified the association between the overexpression of RRM2 and poor outcome of Luminal A breast cancer subtype. In this study, we hypothesize that silencing RRM2 using siRNA might strip Luminal A breast cancer subtype off malignant characteristics. This study aimed to investigate the therapeutic potential of silencing RRM2 using siRNA treatment to Luminal A cell lines in vitro and check the efficacy of combination therapy of targeting RRM2 and current standard agents based on the previous results.

We have found that targeting RRM2 reduces malignance of Luminal A cell lines and shows synergistic effect with Tamoxifen or Doxorubicin in the inhibition of MCF 7 cell line.
Furthermore, we expect that these experimental outcomes can be used to develop a novel strategy based on gene expression profiling to optimize treatment for Luminal A breast cancer patients.
MATERIALS AND METHODS

Patient samples

Eight hundred and twenty cases of formalin-fixed and paraffin-embedded clinical samples were obtained from the Samsung Medical Center (Seoul, Korea).

RNA expression of RRM2 in clinical study

RNAs from the FFPE samples were isolated by tissue preparation system (Siemens AG, Munich, Germany) and qRT-PCR was performed using LightCycler 480 (Roche Applied Science). Results of the qRT-PCR were expressed as Ct values. Ct value for UBE2C was normalized as relative expression value (△Ct value) using three reference genes (CTBP1, CUL1, and UBQLN1)

\[ \triangle Ct_{target} = \frac{(Ct_{CTBP1}+Ct_{CUL1}+Ct_{UBQLN1})}{3} - Ct_{target} \]

Cell culture and drug treatment

The immortal non-tumorigenic epithelial MCF-10A and Luminal A breast cancer cell lines were purchased from ATCC (American Type Culture Collection). MCF-10A was maintained in MEBM along with the additives that can be obtained from Lonza, Basel, Switzerland as a kit.

Luminal A breast cancer cell lines were cultured in appropriate media such as Dulbecco's High Glucose Modified Eagles Medium (DMEM) (for MCF 7 and T47D) and RPMI 1640 (for ZR 75-1 and HCC1428) supplemented with 10% Fetal Bovine Serum (FBS), 1% Penicillin and Streptomycin (GE Healthcare life sciences Hyclone, Utah, USA)

Dimethyl Sulfoxide (DMSO), Doxorubicin and Tamoxifen was purchased from Sigma-Aldrich Inc., MO, USA. For drug treatment, cells were treated with DMSO (10-5 %) as vehicle control, or varied concentrations of Doxorubicin (0.5; 0.75; 1;
1.25 μg/ml), or varied concentration of Tamoxifen (5; 7.5; 10; 12.5; 15; 20 μM).

**SiRNA and transfection**

Inhibition of RRM2 expression in Luminal A breast cancer cell lines was performed by siRNA. Both non-specific control siRNA and RRM2 siRNA were designed, synthesized and purified by GE Healthcare Dhamacon and stored at -20°C. When cell density reaches 50-60%, the RRM2 siRNA or non-specific control siRNA was transfected using DhamaFECT™ transfection reagent (GE Healthcare Dhamacon, Inc, Lafayette, CO, USA) according to the manufacturer’s instructions. Six hours after transfection, the medium containing transfection reagent was removed, and 24h later, cells were subjected to the following assays. Non-specific siRNA was used as a negative control. ON-TARGETplus SMARTpool siRNA targeting RRM2 with 4 sequences (5’-GGA GUG AUG UCA AGU CCA A-3’; 5’-GCG AUG GCA UAG UAA AUG A-3’; 5’-CCA CGG AGC CGA AAA CUA A-3’; 5’-GAA UUG CAC UCU AAU GAA G-3’) were used to knockdown RRM2 expression. The transfection efficiency was tested by Western blot analysis.

**Western blot analysis**

For Western blot analysis, cells were harvested and lysed with RIPA buffer [150Mm NaCl, 10Mm Tris (pH 7.2), 0.1% sodium dodecyl sulphate (SDS), 1% Triton X-100, 1% deoxycholate and 5mM ethylene diaminetetraacetic acid (EDTA)] supplemented with Phosphatase inhibitor and Protease inhibitor cocktail (Roche prognostics, Mannheim, Germany) and then sonicated for 5 seconds at room temperature using Brandson Digital Sonifier 450 at 230V and 60Hz. Disrupted cell suspension were centrifuged at 14,000 rpm at 4°C for 15 min to separate protein lysate from cell debris. The protein concentration was measured using bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Rockford,
IL, USA). The protein samples were boiled in 1X SDS sample buffer for 10 min for complete denaturation. Equal amounts of protein from each group were loaded into a 12% SDS-polyacrylamide gel electrophoresis (PAGE) and then electrotransferred onto Polyvinyl idene fluoride (PVDF) membrane, which were blocked with 5% skim dry milk in 1X TBST (Tris-buffered saline with 0.1% Tween-20) and incubated with primary antibody at the appropriate final concentration followed by hybridization with horseradish peroxidase-conjugated anti-rabbit, anti-mouse or anti-goat secondary antibodies (1:2000). For each step, the membrane was washed with 1X TBST three times for 10 min. Finally, western blot images were developed on photographic film using enhanced chemiluminescence (ECL) reagent.

**Cell proliferation assay**

Cells were seeded at a density of 50% in 6-well plates and transfected with either RRM2 siRNA or non-specific targeting siRNA. After 24 hours, transfected cells were harvested and reseeded into 96-well plate at 5000 cells/well. Cells were incubated for 24, 48, 72, 96 and 120 h at 37°C in a humidified atmosphere containing 5% CO2, and were imaged using the IncuCyte ZOOM (Essence BioScience, Inc., Ann Arbor, MI, USA). Cell viability was measured using a water-soluble tetrazolium (WST) colorimetric assay (Ez-Cytox, Daeil Lab Service, Seoul, Korea). Absorbance was measured at 450 nm by using a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA).

**Wound healing assay**

In vitro wound healing assay was performed to examine the migration of Luminal A cells transfected with either control siRNA or RRM2 siRNA. Transfected cells were trypsinized and reseeded at 90%-100% of density into ImageLock™ 96-well plate for highly accurate monitoring of cell movement in to
the wounded zone. Wounds were induced by a single scratch on the monolayer of cells using WoundMaker™ device (Essence BioScience, Inc., Ann Arbor, MI, USA). Time-lapse cell motility towards and relative cell density at the scratch site were measured by IncuCyte ZOOM (Essence BioScience, Inc., Ann Arbor, MI, USA)

**Cell colony formation assay**

5000 or 10000 cells transfected with either RRM2 siRNA or control siRNA were seeded into 6-well plate and incubated at 37° c in a humidified atmosphere containing 5% for 2 weeks. The medium was changed every 3 days. After washing twice with PBS, the colonies were stained with crystal violet 0.5% solution (Sigma-Aldrich Inc., MO, USA) for 5 min to visualize formed colonies. After crystal violet being rinsed off, 10% glacial acetic acid (Duksan Pure Chemical Co., Ltd, Gyeonggi-do, Korea) solution was applied to stained colonies and measure at 595 nm using SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA) for quantitative analysis of colony number.

**Cell cycle analysis**

The cell cycle distributions in the infected cells were analyzed using flow cytometer following propidium iodide (PI) staining. Cells were seeded at a density of 50% in 6-well plate. After transfected with either RRM2 siRNA or control siRNA, medium and cells were collected by trypsinization at 24, 48 and 72 h time point. Cells were washed twice with PBS and subjected to overnight fixation with 75% Ethanol (Samchun Chemical Co., Ltd, Seoul, Korea) at 4° c. The ethanol was removed by centrifugation and the remaining cell pellets were treated with 100 µg/ml DNase-free RNase and 10 µg/ml PI solution in PBS and analyzed by flow cytometer (FACS Cali-bur, BD Biosciences, CA, USA) according to manufacturer’s instructions.
Chou and Tatalay analysis

To determine the pharmacologic interaction between RRM2 siRNA and Tamoxifen, Doxorubicin, the Chou and Tatalay analysis was used. The method assesses synergy and antagonism by quantifying the divergence of the combination effect from the expected additive effect of the two therapeutic agents [14]. Dose-effect curves for each regimen and for serial dilutions of each agent or combination of agents were plotted using the median-effect equation. A combination index (CI) was estimated from dose-effect data. CI < 1, = 1, > 1 indicates synergy, additivity and antagonism, respectively.

Annexin V/ PI (Propidium iodide) staining for apoptosis

Cells apoptosis was measured with an Annexin V–FITC apoptosis detection kit (BD Biosciences). Cells was resuspended in binding buffer [10 mM Hepes (pH 7.4), 140 mM NaOH and 2.5 mM CaCl2] at a concentration of 106 cells/ml. Annexin V–FITC (5 µl) and PI (5 µl) were added, vortex-mixed gently and incubated for 15 min at room temperature in the dark. Apoptotic cells were analyzed immediately on a flow cytometer (FACS Cali-bur, BD Biosciences, CA, USA)

Statistical analysis

Univariate Cox’s proportional hazard analysis were performed to assess RRM2 and survival in luminal A breast cancer. A P value < 0.05 was considered statistically significant.

For statistical analysis between two groups, Student's t-test for independent means was applied. The differences between the any two groups out of several groups were analyzed by two-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons. Statistical analysis was performed with the Graphpad Prism
version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. A value of $P < 0.05$ was considered as statistically significant.
RESULTS

Correlation between RRM2 overexpression and poor outcome in Luminal A breast cancer

Of the 820 patients of breast cancer, 410 (50%) were classified as Luminal A, 112 (13.7%), Luminal B, 192 (23.4%), HER2, 105 (12.8%) and Triple-negative breast cancer (TNBC) (Table 1). Characteristics of luminal A patients were summarized in Table 2.

Univariate Cox’s proportional hazard analysis identified RRM2 differentially expressed in Luminal A patient database between good and poor outcome group. As a result, RRM2 high expression was significantly associated with poor OS (overall survival) (HR = 1.39, 95% CI: 1.14-1.7, \( P = 0.001 \)), poor DFS (disease free survival) (HR = 1.45, 95% CI: 1.2-1.75, \( P = 0 \)) and poor DMFS (distant metastasis free survival) (HR = 1.39, 95% CI: 1.14-1.69, \( P = 0.001 \)) in Luminal A subtype (Table 3). In Luminal B subtype, we also observed the same pattern of this correlation. However, in HER2 and TNBC, we did not see significant association between RRM2 expression and poor outcome.

RRM2 is overexpressed in Luminal A breast cancer

The expression level of RRM2 was determined in immortalized non-transformed (normal) breast cell line (MCF10A) and a panel of 4 Luminal A breast cancer cell lines (MCF-7, ZR 75-1, T47D and HCC1428) at protein level (Figure 1). To adjust the same amount of protein used for analysis, \( \beta \)-actin was employed as an internal quantitative control. The expression level of RRM2 of Luminal A breast cancer cell lines were increased remarkably compared to it of normal cell line. MCF10A cells exhibited almost no protein expression of RRM2, whereas 4 Luminal A cell lines showed strong protein expression.
**RRM2 knockdown inhibits cell proliferation**

We first knocked down RRM2 expression in MCF-7 and ZR 75-1 cells by transfecting with RRM2 siRNA (10nM). Compared to non-targeting siRNA transfected cells, the cells transfected with RRM2 siRNA had significantly reduced RRM2 protein expression level, indicating that RRM2 siRNA has a high knockdown efficiency (**Figure 2A**). Next, we checked the cell proliferation and growth using WST assay (**Figure 2B**) and colony formation assay (**Figure 2C**) following the transfection of MCF7 and ZR 75-1 cells with RRM2 siRNA. The results demonstrated that RRM2 siRNA inhibited the proliferation and clonogenicity of MCF7 and ZR 75-1 cells effectively.

**RRM2 knockdown induces G0/G1 arrest in cell cycle**

To examine whether silencing RRM2 leads to cell cycle arrest, the phase distribution of the cell cycle was analyzed by flow cytometry. MCF7 and ZR75-1 cells transfected with RRM2 siRNA were found mostly in the phase G1 compared with control siRNA transfected cells. Flow cytometry analysis revealed significant increases from 50.38% to 70.29% and 59.96% to 69.70% after 24 h, 53.38% to 75.56% and 65.66% to 80.13% after 48 h and 55.56% to 79.62% and 72.23% to 77.65% after 72 h in G1 population in MCF 7 and ZR75-1, respectively (**Figure 3**)

**RRM2 knockdown reduces wound healing ability**

Next, the effect of knockdown of RRM2 expression on the wound-towards-migration property of Luminal A breast cancer cells was assessed. The wound healing assay confirmed the reduced movement pattern onto scratch wound site of MCF7 and T47D cells transfected with RRM2 siRNA for 48 h and 72h, respectively, as compared to control siRNA- transfected cells (**Figure 4 A,B**)
Combination therapy enhances the cytotoxic effects of endocrine therapeutic and chemotherapeutic

First, dose-effect curves for each drug alone were established (Figure 5A) Next, to investigate whether co-treatment of Luminal A cells (MCF7) with RRM2 siRNA and endocrine (Tamoxifen) or chemotherapeutic agent (Doxorubicin) shows synergistic effect on the inhibition of cell proliferation, cells were transfected with RRM2 siRNA for 24 h before being exposed to low concentration of Tamoxifen (10 μM) or Doxorubicin (0.5 μg/ml) for 2 more days. Remarkably, cells exposed to drug in the presence of RRM2 siRNA suffered significant cell growth inhibition compared to cells exposed to drug alone (Table 4). In detail, an enhanced apoptotic effect was observed in the co-treatment regimen of RRM2 siRNA and Tamoxifen through Annexin V/PI staining assay (Figure 5B) or RRM2 siRNA and Doxorubicin through increased level of cleave PARP (Poly (ADP-ribose) polymerase) (Figure 5C)
Figure 1. Expression level of RRM2 at protein level.

Lysates of indicated cell lines were subjected to SDS-PAGE following western blot analysis using specific antibody. For a quantitative control, anti β-actin antibody has been used. INF: Immortalized non-transformed human breast epithelial cell line.
Figure 2. RRM2 knockdown reduces proliferation and growth of MCF7 and ZR 75-1 cells.

(A) Efficiency of RRM2 knockdown by RRM2 siRNAs was measured by Western blot. GAPDH (~36kDa) was used as internal control. (B) The proliferation of MCF7 and ZR75-1 cells was determined by WST assay after transfection with RRM2 siRNA. Data were presented as mean ± SEM (n = 3). (C) The growth of MCF7 and ZR 75-1 cells was determined by colony formation assay (n = 3). *** P < 0.001
Figure 3. RRM2 knockdown triggers cell cycle arrest at G0/G1 phase in MCF7 and ZR75-1 cells.

Cells were infected with RRM2 siRNA for 24 h, 48 h and 72 h. RRM2 knockdown induced a significant increase in cells arrested in the G1 phase.
Figure 4. RRM2 knockdown suppresses migration of MCF7 and T47D cells. (A) MCF7 and T47D cells were transfected with either RRM2 siRNA (10nM) or control siRNA (10nM) and reseeded with same number of cells. Cells movement towards scratch-induced wounds were observed after 48 h (for MCF7) and 72 h (for T47D). Representative pictures of the wounds at random locations were captured. (B) Relative cell density at scratch wound site.
Figure 5. Enhanced effect in inhibition of MCF 7 resulting from combination therapy.

(A) Kill curve of single therapy (Tamoxifen, Doxorubicin and RRM2 siRNA) for MCF7. A number of 5 x 10^4 cells or a cell density of 50% were seeded into 12-well plate. After 24 h incubation, cells were treated with Tamoxifen or Doxorubicin for 48 h or RRM2 siRNA for 72 h. Cell viability was determined by WST assay. (B) Co-treatment of RRM2 siRNA and Tamoxifen enhances apoptosis. Cells were seeded into 6 well-plate at a density of 50%, 24 h after transfection with RRM2
siRNA (40nM), transfection medium was changed into complete medium containing Tamoxifen (10µM), cells were incubated for further 48 h before being subjected to Annexin V PI staining to assess cell apoptosis. (C) 24 h after transfection with RRM2 siRNA (40nM), transfection medium was changed into complete medium containing Doxorubicin (0.5 µg/ml), cells were incubated for further 48 h before being subjected to Western blot to compare protein level of cleaved PARP and qRT-PCR to confirm knockdown efficiency.
Table 1. Patient classification into 5 different subgroups based on Hormone status.

<table>
<thead>
<tr>
<th>Definition</th>
<th># of samples</th>
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<tr>
<td>Luminal B</td>
<td>112</td>
</tr>
<tr>
<td>HER2</td>
<td>192</td>
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<tr>
<td>TNBC</td>
<td>105</td>
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<td>Unknown</td>
<td>1</td>
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<tr>
<td>Total</td>
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Table 2. Clinical characteristics of breast cancer patients.

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<td></td>
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<tr>
<td></td>
<td>2</td>
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Abreviations : NPI, Nottingham prognostic index
Table 3. Diagnostic significance of RRM2 expression in Breast cancer.

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<th>DFS</th>
<th>OS</th>
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<td></td>
<td>DMFS</td>
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<tr>
<td></td>
<td>lower 95%</td>
<td>upper 95%</td>
<td>p-value</td>
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<tr>
<td>All</td>
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<td>1.34 0.036</td>
<td>0.01</td>
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<tr>
<td></td>
<td>1.39 1.14</td>
<td>1.69 0.001</td>
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<td>1.53 1.08</td>
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<td>0.417</td>
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<tr>
<td>TNBC</td>
<td>0.88 0.64</td>
<td>1.2 0.431</td>
<td>0.417</td>
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DMFS: Distant metastasis free survival; DFS: Disease free survival; OS: Overall survival
Table 4. Chou Tatalay drug interaction analysis of RRM2 siRNA and Tamoxifen or Doxorubicin.

<table>
<thead>
<tr>
<th>siRRM2 (nM)</th>
<th>Tamoxifen (µM)</th>
<th>Doxorubicin (µg/ml)</th>
<th>Cytotoxicity (%)</th>
<th>CI</th>
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Combination index (CI) < 1 indicates synergism.
DISCUSSION

Previous study has demonstrated prognostic significance of RRM2 in estrogen-negative breast cancer [15]. Collectively, RRM2 has been gaining more importance as a prognostic factor in breast cancer.

Drug resistance is an emerging barrier that limits the effectiveness of endocrine or chemotherapy [16]. Subsequent development of drug resistance can result in rapid disease progression during or shortly after completion of cancer treatment. The mechanism of resistance to 'classical' cytotoxic chemotherapeutics and to therapies that are designed to be selective for specific molecular targets share many features, such as alterations in the drug target, activation of pro-survival pathways and ineffective induction of cell death [17].

Tamoxifen is prescribed as an adjuvant therapy in early breast cancer [18]. It improves overall survival, and is thought to have made a significant contribution to the reduction in breast cancer mortality over the last decades [19]. However, despite an initial response, up to 50% of ER+/PR- (ER/progesterone receptor – positive) tumors, 66% of ER+/PR- cases, and 55% of ER-/PR+ cases fail to respond or develop early resistance to tamoxifen and eventually relapse with more aggressive tumors [20] [21].

In this study, RRM2 overexpression was identified to be related to poor outcome (DMFS, DFS and OS) in Luminal A patient subgroup, suggesting that it appears to be a promising prognostic biomarker to distinguish positive and negative outcomes in Luminal A subtype. Functional experiments using siRNA to selectively silence RRM2 expression, we have revealed that down-regulation of RRM2 leads to inhibition in cell proliferation, cell growth, wound healing ability and triggers cell cycle arrest at G1 phase. These findings were consistent with previous study suggesting that knockdown RRM2 expression reduces cell motility and cell growth through AKT overexpression [22]. Together, targeting RRM2 seem to hold
significant therapeutic potential in treating Luminal A breast cancer subtype.

Furthermore, this study demonstrated that silencing RRM2 improve responsiveness of MCF 7 to Tamoxifen and Doxorubicin (Table 4). Synergistic inhibition of MCF7 by combination of RRM2 siRNA and Tamoxifen is due to enhanced apoptotic effect as shown in Figure 5B. The mechanism of synergy might be explained by the possibility of treatment of MCF7 with RRM2 siRNA blocking cell transition into S phase and facilitating the anti-proliferative effect of Tamoxifen. Co-treatment of RRM2 siRNA and Doxorubicin was shown to increase expression level of cleaved Poly (ADP-ribose) polymerase (PARP), characterizing cells undergoing apoptosis [23, 24].

These findings lead us to suggest the combination therapy of targeting RRM2 and endocrine or chemotherapy to Luminal A subtype patients who have an elevated RRM2 expression followed by gene expression profiling test in the purpose of optimizing treatment regimen, preventing drug resistance resulting from low drug concentrations and risk of relapse.
REFERENCES


ABSTRACT IN KOREAN

리보뉴클레오틱idot 류단백 M2 (RRM2) 유전체는 리보뉴클레오틱idot 환원 효소를 구성하는 두개의 서브 유닛 중 하나로써 ribonucleotide 5'-diphosphate를 2'-deoxyribonucleotide로 환원시키며 DNA 합성 과정에서 꼭 필요한 효소이다. 
RRM2의 과발현은 종양의 악성화 그리고 재발의 위험성과 연관이 있으며, 심지어 치유가 쉬운 유방암 서브타입인 Luminal A에서도 관련이 있다. 본 연구에서는 RRM2를 Luminal A 유방암 표적치료제의 타겟으로서의 가능성을 확인하였다. Luminal A 세포주에서 small interfering RNA에 의한 RRM2의 합성 억제는 세포 성장, 클론형성, 그리고 상처 치유 능력이 줄어드는 경향을 보였고 G1 phase를 활성화하는 억제를 보였다. MCF-7 cell line에서 RRM2를 타겟하는 siRNA를 Tamoxifen 또는 Doxorubicin과 함께 처리하였을 때 시너지 효과가 있는 것으로 보아 RRM2는 치료의 타겟이 될 수 있음을 확인하였다. 이 연구는 RRM2 발현성이 높고 예후가 안좋은 Luminal A 환자에서 RRM2의 억제가 치료전략으로서 가능성이 있음을 보여주며, 또한 endocrine 또는 chemotherapy와 같이 combined therapy로 처리할 시에 더 좋은 치료 효능을 기대할 수 있음을 보여준다.

주요어 : Luminal A breast cancer; Ribonucleotide reductase M2 subunit; small interfering RNA; Synergistic effect
학번: 2014-22150