MicroRNA-dependent regulation in the preceding step of prostate cancer

Yoon Jin Cha

Department of Medicine

The Graduate School, Yonsei University

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Directed by Professor Nam Hoon Cho

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Yoon Jin Cha

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This certifies that the Doctoral Dissertation of Yoon Jin Cha is approved.

Thesis Supervisor : Nam Hoon Cho

Thesis Committee Member#1 : Kyung-Sup Kim

Thesis Committee Member#2 : Young Deuk Choi

Thesis Committee Member#3: Yun-Han Lee

Thesis Committee Member#4: Han Suk Ryu

The Graduate School
Yonsei University

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ABSTRACT

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Yoon Jin Cha

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Background: Prostatic intraepithelial neoplasia (PIN), convincingly acceptable precursor lesion of prostate cancer (PCa), is characterized by obvious cytologic atypia in luminal cells with preserved basal cells. However, molecular association between PIN and PCa has been vaguely clarified. We aimed to identify miRNAs and surrogate target mRNA specific to regulate development of PIN and PCa, and to identify clinical implication of PIN as precancerous lesion of PCa.

Materials and methods: Among the 388 radical prostatectomy patients, 69.3% harbored PIN, and large PIN was observed in 56 patients. Clinicopathologic analysis was performed based on the PIN status. To anlaysis miRNAs and surrogate target mRNAs, PIN clusters were obtained by macrodisseciton or laser capture microdissection from formalin-fixed paraffin embedded tissue. Using miRNA microarray screening analysis for each PIN or PCa to compare with normal prostatic tissue, top-ranked miRNAs with relatively lower expression were selected. Immunohistochemistry for FGFRL1, BACH1, ephrin-A3, STAT3, and ZEB1was performed, and expression level of the proteins in PCa, PIN, and normal prostatic tissue was analyzed.

Results: Patients harbored PIN showed significant less lymphovascular invasion, less lymph node metastasis, lower tumor volume, lower Gleason score, lower death rate, longer overall survival compared to patients without PIN. Significant downregulation of miR-155, miR-210, miR-153, and miR-200c was observed. Subsequent validation step using

immunohistochemistry against the candidate gene products revealed significant high expression of STAT3, ephrin-A3 and ZEB1 in PCa compared to PIN and normal prostatic tissue. Significant stepwise increase in expression of STAT3 and ZEB1 was observed from normal prostatic tissue to PCa.

Conclusion: More favorable clinicopathologic parameters and longer overall survival in patients with PIN imply disease progression from PIN to PCa. Furthermore, downregulation of cancer-related miRNAs - miR-155, miR-210, miR-153, and miR-200c- in both PIN and PCa and stepwise increased expression of STAT3 and ZEB1 support that PIN is a preceding lesion of PCa and early carcinogenesis starts at the molecular level.

Key words : Prostatic intraepithelial neoplasia, Prostatic neoplasms, MicroRNAs, STAT3, ZEB1

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I. INTRODUCTION

Prostate cancer (PCa) is 5th common carcinoma in Korean men,[1] the most common in American men, and one of cancer related leading cause of death.[2] Detection of PCa at early stage is important because of excellent patients prognosis at early stage compared to the progressed state. With aid of routine PSA (prostate-specific antigen) screening and subsequent needle biopsies, detection rate of early PCa is increasing.

As precursor of PCa, prostatic intraepithelial neoplasia (PIN) is most accepted candidate as preinvasive malignant lesion. Several studies showed that detection rate of carcinoma was higher in subsequent rebiopsies in patients having PIN in initial needle biopsies than patients without PIN in initial biopsies.[1,3]

Histologically, PIN is characterized by atypical epithelial proliferation in preexisting duct with intact basal cells, having four common morphologic

patterns including tufting (87%), micropapillary (85%), cribriform (32%) and flat (28%) patterns.[4] Additional to its cytologic atypia like PCa, PIN has predilection in peripheral zone which is vicinity of carcinoma.[5]

Genetically, PIN demonstrates loss of chromosome 8p, which is also found in carcinoma.[6] TMPRSS2 gene alteration is observed in 62% of PCa, 17% of PIN, and none of normal prostate.[7] There have been many studies of genetic alteration in tumor progression and metastasis of PCa, studies of genetic relationship between PIN and PCa are relatively rare. [6-8] MicroRNAs (miRNAs) are small non-coding RNAs that regulate the expression of mRNAs at post-transcriptional level. According to the targeted mRNA, miRNAs can act either oncogenic or tumor suppressive. In PCa, miRNAs influencing androgen signaling and cancer cell invasion and migration have been thoroughly studied to develop the therapeutic targets or biomarkers for prognosis. [9] However, miRNA state of PIN and its correlation of miRNA state of PCa has not been studied.

In present study, we investigated clinical implication of PIN as precursor of PCa. We also aimed to verify the expression level of miRNAs in PIN and matched PCa, compared to normal prostatic tissue, and to find the target mRNAs and expression of related proteins involving the early carcinogenesis of PCa.

II. MATERIALS AND METHODS

1. Patient selection and clinicopathologic evaluation

Four hundred and twenty five radical prostatectomies were performed due to prostate cancer between 2005 and 2008 in Severance Hospital. Thirty-seven patients who had neoadjuvant anti-androgen therapy were excluded. As result, 388 radical prostatectomies comprised the population of this study. From medical chart review, clinical parameters including age, PSA level, overall survival and follow up period were recorded. For pathologic evaluation, after radical prostatectomy, the whole prostate specimen was made into whole mount slides. The specimen was fixed in buffered formalin (10%) after macroscopic examination. The apex of prostate was obtained by conization, and vertically sectioned to examine the bladder neck invasion. The basal region was shaved to evaluate the surgical margin. The main body was serially sectioned vertically to the urethra into 5-mm-thick slice from the apex to the base. The whole sectioned pieces were pictured and fully embedded. Each paraffin block was cut into 4-μm-thick slices and stained with hematoxylin and eosin for a microscopic examination. Whole mount slides of prostates were reviewed by two pathologists (NHC and YJC) for Gleason score, pathologic T stage, lymph node metastasis, perineural invasion, lymphovascular invasion, tumor volume. Grid method [10] was used for measuring the tumor volume. Either the presence or absence of PIN was reviewed for each patient. PIN was categorized, in more detail, as follows; absence of PIN, presence of small PIN,

and presence of large PIN. Large PIN was defined well-defined lesions absolutely composed of high grade PINs at least more than 2 mm^2 (one x100 magnification field) (Fig 1A).

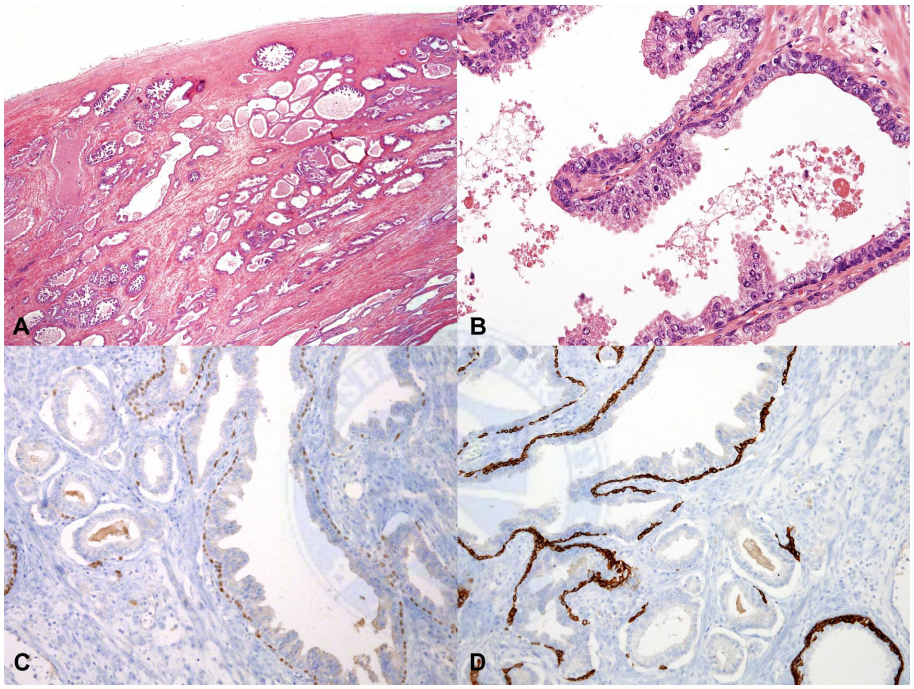

Figure 1. Grouped prostatic intraepithelial neoplasia (PIN) and comfirmation with immunohistochemistry. (A) Under low power magnification, grouped PIN is well delineated (hematoxylin and eosin, x12.5). (B) The lesion is composed of atypical luminal epithelial cells with intact basal cell layer (hematoxylin and eosin, x100). (C and D) P63 and high molecular weight cytokeratin (HMWCK) immunohistochemical stainings reveals intact basal cell layer of PIN, compared to the adjacent carcinoma. (C, p63, x100, D, HMWCK, x100)

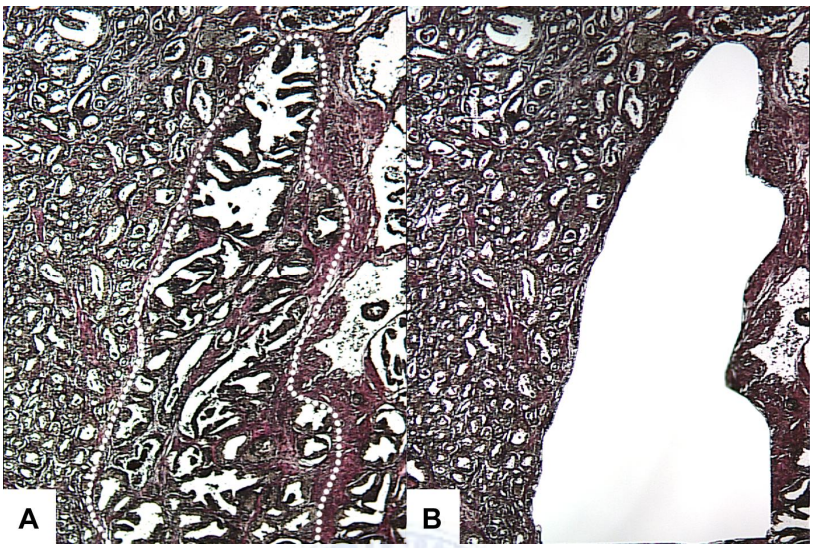

Figure 2. Laser capture microdissection for prostatic intraepithelial neoplasia.
Before (A) and after (B) of laser capsure microdissection of prostatic intraepithelial neoplasia in the area of interemixed PIN and prostate cancer

2. Tissue sampling of PIN by laser caputure microdissection and macrodissection from formalin-fixed, paraffin-embedded tissue

PIN is pathologically characteristic of atypical luminal cells sufficient for prostate cancer but with preservation of basal cells (Fig. 1B). Multifocal isolated or clusters of PIN were confirmed by loss of basal cells using both p63 nuclear stain and CK HMW membranous stain restricted to basal cells (Fig 1C and 1D). Immunohistochemically-proven PINs were removed by laser capture microdissection (Fig 2).

Microdissection of PIN was performed by Leica LMD7000 (Leica Microsystems, Wetzlar, Germany) from FFPE tissue. 5-μm-thick paraffin sections were stained with hematoxylin and eosin, mounted on crosslinked polyethylene (PEN) foil attached to a carrying frames. After defining PIN area, ultraviolet laser cut the edge around PIN on the foiled slides. The laser beam was moved by optics to ensure the precision. The microdissected tissue was transported by gravity without contact by pressure of laser beam. To test the efficiency of DNA isolation, 50–2000 cells were microdissected.

Macrodissection was also performed to obtain large grouped PIN, by using sharp 24-gauaged needle after dripping xylene directly on cover-glass removed slide.

3. MicroRNA microarray and prediction of putative targets of miRNAs

Ten specimens including large grouped PIN were selected for miRNA preparation. Paired PIN and PCa were obtained from FFPE tissue in each patient by laser capture microdissection and macrodissection. Benign prostatic tissue was obtained from transurethral resection of prostate of 2 patients having nodular hyperplasia for reference control. miRNA was separated from minimum 400 ng of total RNA using the PANArray™ miRNA expression profiling kit following the company's protocol. Fluorescein-labeled 135 cancer-related miRNA probes were used and signal intensities were measured as previously described.[11] Raw data were normalized by intensity of RNU6B in each sample,

and analyzed in Genepix 4000B scanner and PANAGENE software.

Compared with normal prostatic tissue, miRNAs showing more than 2, less than 0.5 fold of intensity signal in PIN and PCa were selected. For each miRNA, miRanda (http://www.microrna.org/microrna/home.do), TargetScan (http://www.targetscan.org/vert_61/), miRBase (http://www.mirbase.org) were used to predict putative targets. Additionally, prior studies about the each miRNA related cancer and/or prostate were reviewed.

4. Immunohistochemical staining and interpretation

Available immunohistochemical staining for FFPE tissue was performed with antibodies against FGFRL1 (Abcam, ab95940, 1:100), BACH1 (Novus, NBP2-01904, 1:300), Ephrin-A3 (Novus, NBP1-19540, 1:100), STAT3 (Santacruz, sc-8019, 1:100), and ZEB1 (Cell signaling, D80D3, 1:100). Briefly, 4-μm-thick paraffin sections were deparaffinized and rehydrated by xylene and alcohol solution. Immunohistochemistry was performed using the Ventana Discovery XT automated stainer (Ventana Medical System, Tucson, AZ, USA). Antigen retrieval was performed using CC1 buffer (Cell Conditioning 1; citrate buffer Ph 6.0, Ventana Medical System). Appropriate positive and negative controls for immunohistochemistry were included. Immunohistochemical staining of all cases were assessed by two pathologists (YJC and NHC), using light microscopes. Nuclear staining for BACH1, and cytoplasmic staining for FGFRL1, Ephirin-A3, and STAT3 were considered as positive. Interpretation of

immunohistochemical staining was determined by multiplying the proportion of stained cell (0% = 0, 1-5% = 1, 6-25% = 2, 26-50% = 3, 51-100% = 4) with the immunostaining intensity (negative = 0, weak = 1, moderate = 2, strong = 3).[12]

5. Statistics

Data were analyzed with SPSS for Windows (version 18.0; SPSS Inc., Chicago, IL, USA). For analysis of numerical variables in three subgroups of patients, one-way ANOVA test and Kruskal-Wallis test were performed. Chi-square test was used for comparison of categorical variables. Friedman test and Wilcoxon signed rank test were performed to analyze immunohistochemical staining score. Kaplan-Meier survival curves and log-rank statistics were employed to evaluate time to survival. $P<0.05$ was considered significant.

III. RESULTS

1. Clinicopathologic profile based on PIN status

Of total 388 patients, mean age was 63.45±7.53 years (range 38-84 years), mean PSA level was 14.36±40.51 mg/dL (range 2.0-726.6 mg/dL), mean follow up period was 80.54±18.69 months (range 0.8-112.4 months). When 388 patients were separated into 3 groups according to the PIN status, 119 (30.7%) had no PIN, 213 (54.9%) had small PIN, and 56 (14.4%) had large PIN over 2 mm^2 in maximal dimension. Patients with PIN had significantly

lower serum PSA (p=0.003), lower Gleason score (p=0.007), lower invasive tumor volume (p=0.001), less lymphovascular invasion (p=0.046), less lymph node metastasis (p<0.001), and less death rate (p=0.03). No significant difference was found in patient age, pathologic T stage, and perineural invasion. When patients with small PIN and large PIN were compared, there was no significantly different parameter. The clinicopathologic profile is summarized in Table 1. Overall survival was significantly shorter in patients without PIN (p<0.001). (Fig. 3)

Table 1. Clinicopathologic profiles of patients

	Absence of PIN (n=81)	Presence of small PIN (n=191)	Presence of large PIN (n=41)
Age (mean, years)	64.44±7.30	62.89±7.80	63.46±6.85
Serum PSA (mean, mg/dL) *, †, ‡	25.06±71.58	10.06±8.90	8.40±5.118
Gleason score *, †, ‡	7.13±1.21	6.80±0.95	6.73±0.65
Volume of invasive carcioma (cc) *, †, ‡	4.50±7.06	2.49±3.90	2.18±1.76
Pathologic T stage 2	73 (61.3%)	146 (68.5%)	37 (66.1%)

	Absence of PIN (n=81)	Presence of small PIN (n=191)	Presence of large PIN (n=41)
3	45 (37.8%)	67 (31.5%)	19 (33.9%)
4	1 (0.8%)	0 (0.0%)	0 (0.0%)
Lymphovascular invasion *, †, ‡	16 (13.4%)	14 (6.6%)	2 (3.6%)
Perineural invasion	58 (48.7%)	88 (41.3%)	32 (57.1%)
Lymph node metastasis *, †, ‡	11 (9.2%)	2 (0.9%)	0 (0.0%)
Death *, †, ‡	15 (12.6%)	10 (4.7%)	3 (5.4%)

mean ± standard deviation

* $p<0.05$ among absence of PIN, presence of small PIN, and presence of large PIN

† $p<0.05$ between absence of PIN and presence of small PIN

‡ $p<0.05$ between absence of PIN and presence of large PIN

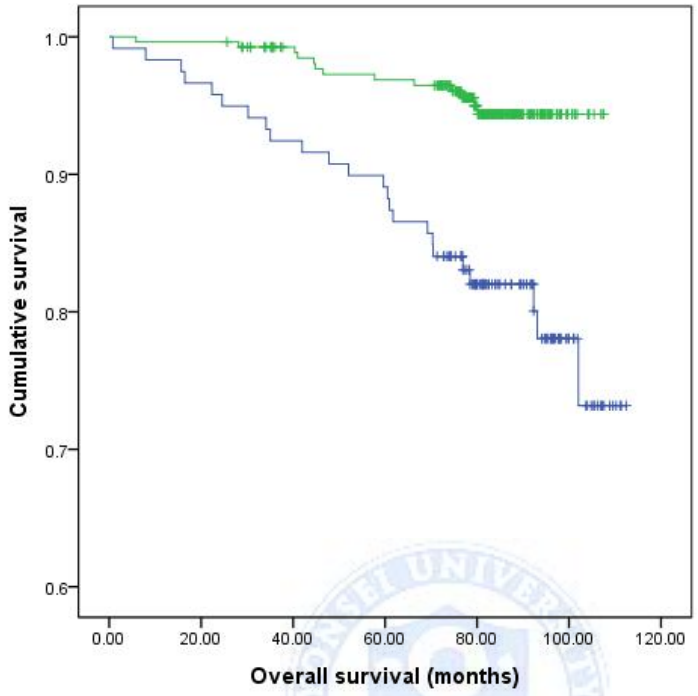

Figure 3. Survival anaylsis of patients with and without prostatic intraepithelial neoplasia (PIN) (green: patients with PIN; blue: patients without PIN)

2. miRNA expression profile and in validation

Twenty nine miRNAs showed detectable alteration in PIN/PCa or PIN/normal prostate among 135 cancer-related miRNA probes-platform array (Fig. 4).

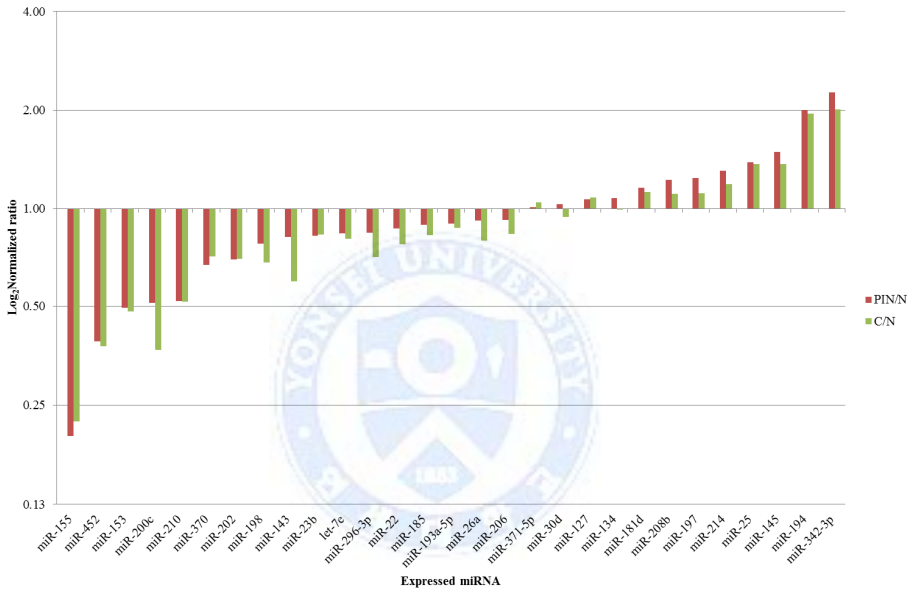

Figure 4. Normalization of miRNA array data (PIN, prostatic intraepithelial neoplasia; PCa, prostate cancer)

Following valid informatics programs, ultimately, four miRNAs were obtained when expression level of PIN was more than or less than 2-folds compared to normal control. MiR-155, miR-153, miR-200c, and miR-210 were significantly decreased in PIN and PCa. (Fig. 5)

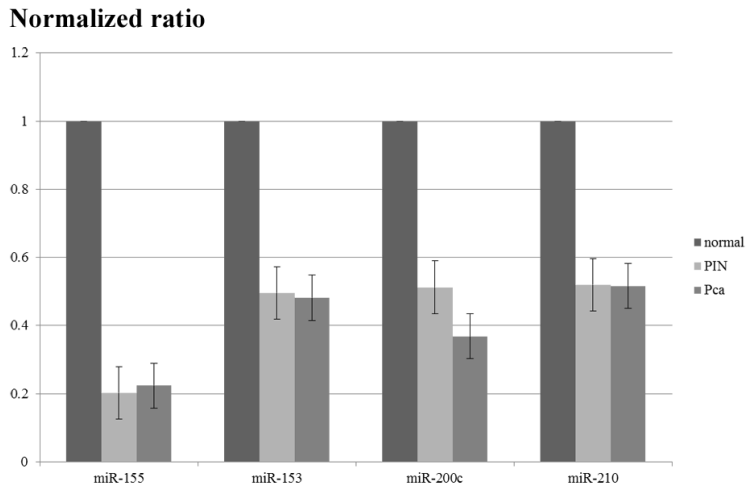

Figure 5. Surrogate candidates of miRNAs with normalized expression ratio between prostatic intraepithelial neoplasia and prostate cancer.

3. mRNA screening specific to miRNA in silico

Four mRNAs and their putative target genes were selected from miRanda (http://www.microrna.org/microrna/home.do), TargetScan (http://www.targetscan.org/vert_61/), miRBase (http://www.mirbase.org), and previous published studies, which are tabulated in Table 2.

Table 2. Selected miRNAs and previously studied targeted genes

MicroRNA	Targeted gene	Role of targeted gene
miR-155	STAT3, SOCS1	Cancer cell proliferation, antiapoptosis, epithelial-mesenchymal transion in breast[13], pancreatic[14], renal cell carcinoma[15]
	BACH1	High N stage, poor clinical outcome in nasopharyngeal cancer[16]
miR-210	FGFRL1	Accelerates cancer cell proliferation by preventing cell cycle arrest in G1/G in poorly differentiated esophageal cancer[17]
	Ephrin-A3	Suppress migration and survivial of endothelial cells[18]
miR-153, miR-200c	ZEB1, ZEB2, SNAI1	Repress E-cadherin expression and promote cancer cell migration and invasion[19]

4. Protein expression level of target mRNA by immunohistochemistry

STAT3 and ZEB1 showed significant stepwise increase of expression in PIN and PCa compared to normal prostatic tissue ($p<0.001$). ZEB1 expression was not found in normal prostatic tissue. Ephrin-A3 expression of PCa was significantly higher than PIN and normal prostatic tissue ($p<0.001$), whereas no signficant difference was found between PIN and normal prostatic tissue. FGFRL1 expression was significantly decreased in PIN ($p=0.006$) and PCa ($p=0.002$) than normal prostatic tissue , but showed no significant difference between PIN and PCa. BACH1 expression was significantly decreased in PIN than PCa ($p=0.003$) and normal prostatic tissue ($p<0.001$) (Fig. 6). The result of protein expression scoring by immunohistochemistry and changes of expression level in PCa, PIN, and normal prostatic tissue are shown in Table 3 and Table 4.

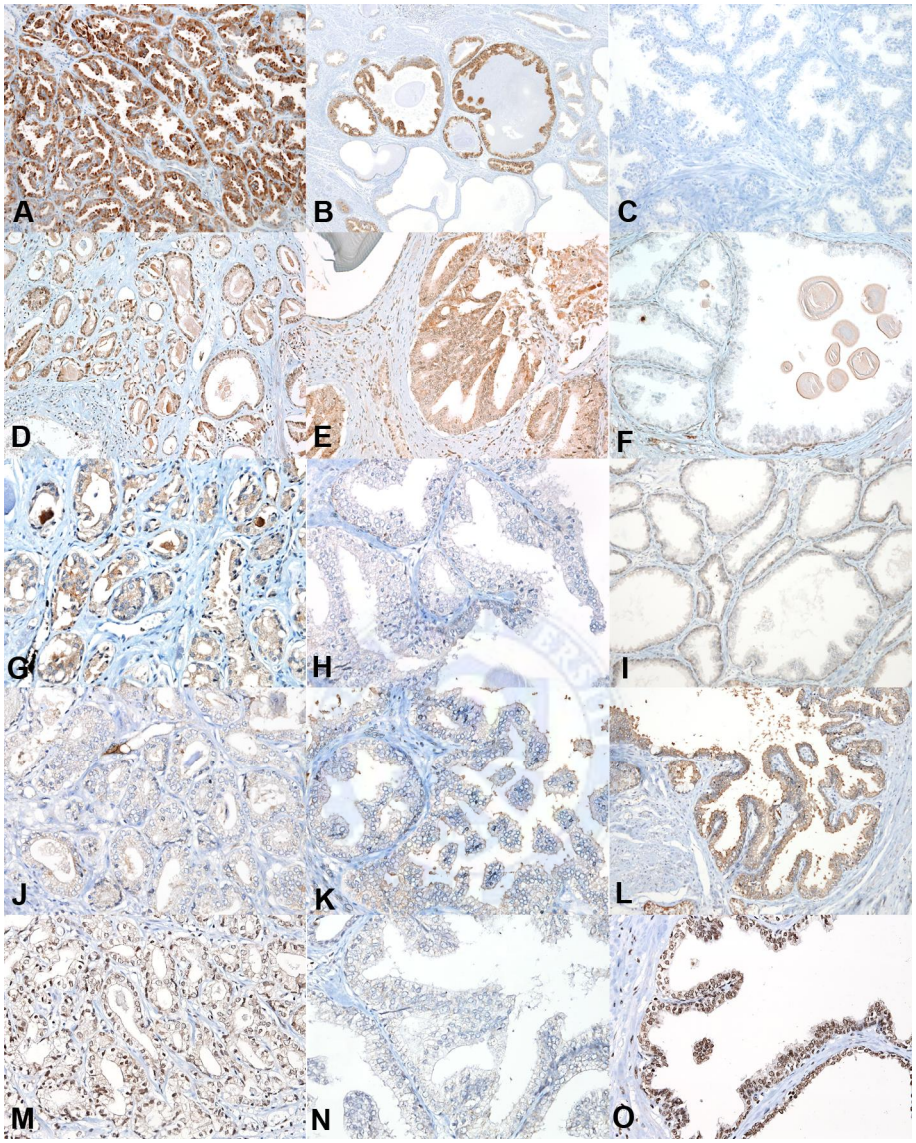

Figure 6. Protein expression by immunohistochemistry applied to prostate cancer, prostatic intraepithelial neoplasia, and normal prostatic tissue. Prostate cancer (first column), prostatic intraepithelial neoplasia (second column), normal prostatic tissue (third column). STAT3 (A, B, C), ZEB1 (D, E, F), ephrin-A3 (G, H, I), FGFRL1 (J, K, L), BACH1 (M, N, O).

Table 3. Semiquantitatively measured protein expression level of target genes of cancer, prostatic intraepithelial neoplasia, and normal prostatic tissue by immunohistochemistry

Antibody	PCa	PIN	Normal
STAT3 *	4.65±3.56	2.41±2.31	0.75±1.43
ZEB1 *	4.69±2.70	1.48±1.29	0
Ephrin-A3 *	2.51±2.97	1.02±1.70	0.84±1.74
FGFRL1 *	2.28±2.64	2.68±2.63	3.79±2.93
BACH1 *	1.26±1.45	0.58±1.21	1.32±2.15

PCa, prostate cancer; PIN, prostatic intraepithelial neoplasia; N, normal prostatic tissue

* $p<0.05$ by Friedman test

Table 4. Changes in protein expression level of target gene by immunohistochemistry

Protein	PCa versus PIN	PIN versus N	PCa versus N
STAT3	I (<0.001)	I (<0.001)	I (<0.001)
ZEB1	I (<0.001)	I (<0.001)	I (0.031)
Ephrin-A3	I (0.001)	NS	I (<0.001)
FGFRL1	NS	D (0.006)	D (0.002)
BACH1	I (0.003)	D (<0.001)	NS

I indicates expression level that is increased in the first group versus other second group. D indicates decreased expression level in the first group versus other second group. P values in parentheses. NS, not significant; PCa, prostate cancer; PIN, prostatic intraepithelial neoplasia; N, normal prostatic tissue

IV. DISCUSSION

Since patients at early stage of PCa have near 100% of 5-year-survival rate compared to the patients at advanced stage, who have only 28% of 5-year-survivial rate,[20] early detection of cancer is deeply required for the improvement of survival rate in PCa. Prostate intraepithelial neoplasia (PIN), referred to the precursor lesion for the prostate carcinoma, is now accepted as early preinvasive neoplasm, which is only microscopically detectable.[21] The rationale of PIN to be a precancerous lesion include some overlapping signs

in common; PIN demonstrates over 50% rate of coexistence with cancer,[22] predominantly localizes in peripheral zone, and shows the increased incidence with age.[23] In previous study, patients with high grade PIN showed significant association with multifocality of tumor, perineural invasion and biochemical recurrence, whereas patients lacking high grade PIN showed better disease free survival.[24] However, it has been reported that patient with PIN had lower tumor volume,[25] and organ-confined low stage in 84.4% of radical prostatectomy patients.[26] In present study, we confirmed the better prognosis of patients with PIN compared to patients without PIN. Patients with PIN showed more favorable clinicopathologic parameters – lower PSA level, lower Gleason score, smaller tumor volume, less lymphovascular invasion and less lymph node metastasis. Given that PIN is an early lesion, preexisted PIN would be replaced by invasive PCa as disease progresses. It is also correlated with result of current study that patients with PIN showed more favorable clinicopathologic parameters and better overall survival compared to patients without PIN. However, we found no significant difference of pathologic T stage according to the presence of PIN, it would be due to the patient cohort of current study, because most patients had radical prostatectomy were pT2 or pT3.

Recently, several molecular alterations in PCa and PIN have been reported.[8] Chromosomal losses of 8p and gains of 8q are frequently found in both PIN and PCa. One of genetic alteration of PCa, TMPRSS2 gene fusion, is

observed in upto 17% of PIN.[7] However, alteration of miRNAs between PIN and PCa is seldomly studied, [27] and many preexisting studies have been focused on progression and metastasis of PCa, rather than progression of PIN to PCa.[9] Since miRNAs play multifunctional roles and regulate are multifunctional and regulate, specific miRNAs in carcinogenesis or PCa progression have not been established except miR-200 families, which is well known miRNAs involving EMT in many cancers.[28,29]

We found four miRNAs that were decreased in both PIN and PCa compared to benign prostatic tissue, which supports that PIN exists at one end of proceeding carcinogenesis of PCa. As multifunction miRNA, overexpression of miR-155 has been reported in breast, pancreas cancer and lymphoma,[13,14,30] whereas downregulation of miR-155 has been found in melanoma and gastric cancer.[31,32] Of two major target candidates of miR-155, STAT3 and BACH1 showed significant differences in protein expression level among PCa, PIN, and normal prostatic tissue. However, although significant different level was found in BACH1, expression scores of PCa, PIN, and normal protstatic tissue were too low, all were under 2, which would have no practical implication, and should be validated in further study. In contrast, STAT3 showed obvious overexpression in PCa and PIN than normal prostatic tissue, increased gradually towards PCa. Stepwise overexpression of STAT3 suggests that PIN might be on the spectrum of carcinogenesis of PCa. Ni Z et al.[33] showed that STAT3 activation was essential in progression of PCa, and correlated with its malignant potential, also

supports that STAT3 would be one of the important molecules altered in early carcinogenesis and progression of PCa.

Mir-210 is one of the hypoxia-regulated miRs, acts as either oncogene or tumor suppressor.[18] It is upregulated in the chronic hypoxic condition, which would be related to the tumor microenvironment.[34] Ephrin-A3 is one of direct target of miR-210,[18] and increased expression of its family has been reported in hepatocellular carcinoma.[35] FGFRL1 is also regulated by miR-210, and downregulated of miR-210 and increased FGFRL1 have been observed in esophageal squamous cell carcinoma.[17] In current study, FGFRL1 showed decreased expression in both PIN and PCa compared to normal prostatic tissue. However, together with BACH1, expression scores of FGFRL1 showed less than 2 fold although expression score itself showed significant difference. Although FGFRL1 has not been studied in PCa, intermediate molecule is presumed to be existed between miR-210 and FGFRL1. In addition, decreased FGFRL1 has been reported in urothelial carcinoma compared to the normal urothelium,[36] tumor suppressive role of FGFRL1 needs to be further inestigated in PCa. We found that ephrin-A3 was significantly overexpressed in PCa compared to PIN and normal prostatic tissue, corresponded with previous study which suggested that miR-210 directly regulated ephrin-A3.[18] We had discordant result of decreased level of miR-210 in present study compared with previous study in PCa which showed increased level of miR-210.[34] However, this discrepancy might be from the different type of materials used in two

studies. We used 10 patients' radical prostatectomy specimen, including paired PIN and PCa from each patient which were paraffine-embedded tissue. In contrast, previous study used 3 PCa cell lines, which was handled in fresh state, not in paraffin embedded state. Since miR-210 in PCa is yet intensely studied, further large-cohort study is needed to clarify the role of miR-210 in PCa and its pathogenesis.

Mir-153 has been identified tumor suppressor. As downregulation of miR-153 is observed in cells of mesenchymal phenotype, miR-153 would be related with epithelial-mesenchymal transition (EMT).[19] MiR-153 inhibits TGF-β-induced (EMT), and downregulated SNAI1 and ZEB2, acts as tumor surressor in oral cancer,[19] breast cancer,[37] and glioblastoma.[38] Along with these studies, we also observed that miR-153 is down-regulated in both PIN and PCa, which suggests that EMT signal begins at the PIN state, early step of PCa carcinogenesis. Nonetheless, another study of PCa showed that miR-153 was upregulated in PCa, and thought to be potentially oncogenic since miR-153 promoted proliferation of PCa through regulation of cell cycle and AKT signaling pathway, and directly targeted PTEN in PCa.[39] As miR-153 has been rarely studied in PCa, further precise study with expanded cohort is required in PCa.

MiR-200c is a member of miR-200 family, that well-known for their regulating EMT in cancer cells,[29] together with miR-153.[19] MiR-200c targets ZEB1 and ZEB2, which are repressors of e-cadherin, reduce cell motility in

cancer cells.[29] Downregulation of miR-200c is found in various types of cancers, along with reduced e-cadherin expression, and correlates with metastasis and poor prognosis. Puhr M et al.[28] investigated miR-200c in PCa, demonstrated that downregulation of miR-200c was related not only with low e-cadherin level and EMT but also with chemotherapy resistance. In the present study, significant downregulation of miR-200 was found in both PIN and PCa. This result implies that PIN would be already affected by EMT signals through miR-200c, which is also supported by significant expression of ZEB1 in PIN and PCa. Attenuation of basal cells in typical finding of PIN would be also explained by miR-200c downregulation reflecting the initiation of EMT process and invasiveness.

Present study had several limitations. First, quantification of target mRNA was unable to assess due to the limited amount of microdissected PIN. Instead, we validated the protein expression level target genes of selected miRNAs with immunohistochemical staining on whole section, which helps to semiquantitative assess of protein expression level and contrast the expression level clearly among PCa, PIN, and normal prostatic tissue. Second, functional study of interaction between selected miRNAs and targeted mRNAs has not been performed. Since miRNAs have multifunctional role and the degree of interactions with targeted mRNAs would show a great variety, validated result would have assisted the role of miRNAs in carcinogenesis in PCa.

V. CONCLUSION

In conclusion, we investigatved clinicopathological implication of PIN in PCa patients, and further compared the expression rate of miRNAs among the PIN, PCa, and normal prostatic tissue. Presence of PIN was associated with more favorable clinicopathological parameters and longer overall survival. Regarding miRNAs and protein expression, PIN and PCa showed significantly decreased miR-155, miR-210, miR-153, and miR-200c. Ephrin-A3 was significantly increased only in PCa than PIN and normal prostatic tissue, implied it contributed to the malignant characteristics in PCa. Interstingly, STAT3 and ZEB1 showed stepwise overexpression in PIN and PCa. This result suggests that STAT3 and ZEB1 could be the key molecule altered from early step of carcinogenesis in PCa and PIN would be already in process of carcinogenesis as precursor of PCa.

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ABSTRACT

Prostate intraepithelial neoplasia 와 prostate cancer 에서의 microRNA 발현의 차이와 carcinogenesis 에 관여하는 연관된 전사 인자

<지도교수 조 남 훈>

연세대학교 대학원 의학과

차 윤 진

목적: Prostatic intraepithelial neoplasia (PIN) 는 prostate cancer (PCa) 의 전구병변으로 여겨지는데, 조직학적으로 세포학적 이형성과 기저세포의 유지를 특징으로 하며 분자생물학적 기전은 잘 밝혀져 있지 않다. 본 연구에서는 PIN 과 PCa 발생에 관여하는 microRNAs (miRNAs) 와 targeted mRNAs 를 규명하고 임상병리학적 의의를 찾고자 하였다.
연구방법: 388명의 radical prostatectomy 환자 중 69.3% 의 환자에서 PIN 이 관찰되었으며, large PIN 은 56명의 환자에서 관찰되었다. PIN 의 유무에 따라 임상병리학적 인자에 대한 분석을 시행하였다. miRNAs 와 mRNA 의 분석을 위해 large PIN 은 macrodissection, small PIN 은 laser capture microdissection 을 통해 formalin-fixed paraffin embedded tissue 에서 조직을 채취하였다. miRNA microarray 분석에서 각 환자의 PIN 과 PCa 에서 정상 prostate 조직에 비해 발현이 떨어진 miRNAs 를 선정하고, FGFR1, BACH1, ephrin-A3, STAT3, ZEB1 에 대한 면역조직화학 염색을 통해 PCa, PIN, 정상 prostate 조직에서의 단백질 발현을 분석하였다.
결과: PIN 이 있는 환자들은 PIN 이 없는 환자들에 비해 lymphovascular invasion, lymph node metastasis, tumor volume, Gleason score 가 유의하게 낮았으며, 낮은 사망률과 높은 생존기간을 보였다. 4개의 miRNA- mir-155, mir-210, mir-153, and

mir-200c- 가 PCa 와 PIN 에서 낮은 발현을 보였고, 면역조직화학염색을 통한 단백질 발현을 보았을 때, PCa 에서 PIN 과 정상 prostate 조직에 비해 STAT3, ZEB1, ephrin-A3 의 발현이 높이 나타났으며, STAT3 와 ZEB1 은 정상 prostate 조직에서 PCa 로 가면서 단계적으로 발현이 높아졌다.
결론: PIN 이 있는 환자에서 더 좋은 임상병리학적 지표와 긴 생존기간은 PCa 가 PIN 에서 좀더 진행된 병변임을 나타낸다. 또한 PIN 과 PCa 에서 cancer-related miRNAs 의 낮은 발현이 관찰되고, STAT3 와 ZEB1 의 단계적 발현의 증가는 PIN 이 PCa 의 전구병변이며, 초기의 carcinogenesis 에 분자생물학적 기전이 관여한다는 가설을 뒷받침한다.

핵심되는 말: Prostatic intraepithelial neoplasia, Prostatic neoplasms, MicroRNAs, STAT3, ZEB1