# mRNA expression of *CYP17A1, CYP11A1, CYP19A1*, *HSD3B1* and *AKR1C2* in metastatic and non-metastatic prostate cancer patients

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## ABSTRACT

The progression of prostate cancer (PCa) mainly occurs caused by androgens. There is a link between intratumoral steroidogenesis and castration-resistant prostate cancer. This study aimed to determine the mRNA expression of various steroidogenic enzymes (*CYP17A1, CYP11A1, CYP19A1, HSD3B1*, and *AKR1C2*) in metastatic and non-metastatic prostate cancer patients. This study was conducted at the Anatomical Pathology Laboratory and Urologi Division, Department of Surgery, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital, Yogyakarta from September-November 2017. Samples were taken from 30 paraffin blocks with adenocarcinoma of prostate, stained with hematoxylin-eosin (HE) and then classified into metastatic and non-metastatic groups. Samples then underwent deparaffinization procedure and examination of mRNA expression of *CYP17A1, CYP11A1, CYP19A1, HSD3B1, AKR1C2* genes using Real-Time PCR. The mean mRNA expressions of *CYP11A1, CYP17A1, CYP19A1, HSD3B1*, and *AKR1C2* genes in the metastatic adenocarcinoma prostate group were 7.08, 10.11, 3.94, 4.84 and 3.58, respectively. In the non-metastatic group, the mean mRNA expressions of *CYP11A1, CYP17A1*, *CYP19A1, HSD3B1*, and *AKR1C2* genes were 4.62, 9.45, 3.46, 2.68 and 4.92, respectively. The mean of mRNA expression of *CYP11A1, CYP17A1, CYP19A1*, and *HSD3B1* genes were higher in the metastatic group than non-metastatic adenocarcinoma prostate group. However, it was not statistically significant ($p>0.05$). The highest mRNA expression of steroidogenic enzymes was the *CYP17A1* gene. In conclusion, the mRNA expressions of *CYP17A1, CYP11A1, CYP19A1, HSD3B1* were higher in the metastatic prostate cancer patients compared to that in non-metastatic prostate cancer patients but statistically not significant.

## ABSTRAK

Perkembangan kanker prostat (PCa) terutama terjadi karena androgen. Ada hubungan antara steroidogenesis intratumoral dan kanker prostat yang resisten-kastrasi. Penelitian ini bertujuan untuk mengkaji ekspresi mRNA dari berbagai enzim steroidogenik (*CYP17A1, CYP11A1, CYP19A1, HSD3B1, dan AKR1C2*) pada kanker prostat metastatik dan non-metastatik. Penelitian ini dilakukan di Laboratorium Patologi Anatomi dan Divisi Urologi, Departemen Bedah, Fakultas Kedokteran, Kesehatan Masyarakat dan Keperawatan, Universitas Gadjah Mada/Rumah Sakit Umum Pusat Dr. Sardjito dari September-November 2017. Sampel diambil dari 30 blok parafin dengan adenokarsinoma prostat dan diwarnai dengan pewarna hematoxylin-eosin (HE) untuk selanjutnya dikelompokkan menjadi kelompok metastasis dan non-metastasis. Sampel selanjutnya dideparafinisasi dan diukur ekspresi mRNAnya untuk gen-gen *CYP17A1, CYP11A1, CYP19A1, HSD3B1, AKR1C2* menggunakan *Real-Time* PCR. Ekspresi mRNA rata-rata untuk gen *CYP11A1, CYP17A1, CYP19A1, HSD3B1,* dan *AKR1C2* pada kelompok prostat metastasis adenokarsinoma berturut-turut adalah 7,08, 10,11, 3,94, 4,84 dan 3,58. Pada kelompok non-metastatik, ekspresi mRNA rata-rata untuk gen-gen *CYP11A1, CYP17A1, CYP19A1, HSD3B1,* dan *AKR1C2* berturut-turut adalah 4,62, 9,45, 3,46, 2.68 dan 4,92. Rata-rata ekspresi mRNA

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gen *CYP11A1, CYP17A1, CYP19A1,* dan *HSD3B1* lebih tinggi pada kelompok pasien prostat adenokarsinoma metastasis dibandingkan kelompok non-metastatik, tetapi secara statistik tidak signifikan (p> 0,05). Ekspresi mRNA tertinggi dari enzim steroidogenik adalah gen *CYP17A1*. Dapat disimpulkan, ekspresi mRNA dari CYP17A1*, CYP11A1, CYP19A1, HSD3B1* lebih tinggi pada pasien kanker prostat metastatik dibandingkan non-metastatik tetapi secara statistik tidak signifikan.

***Keywords:*** Prostate cancer - CYP17A1 - metastatic - non-metastatic - steroidogenesis, mRNA

## INTRODUCTION

Prostate cancer (PCa) is the 4th most common type of malignancy in men worldwide after skin, lung and colon cancers. According to GLOBOCAN data in 2008, prostate cancer ranked 5th in Indonesia. Based on data from the Indonesian Society of Urologic Oncology in 2011, there were 971 people with prostate cancer in the period 2006-2010 with the level of stage 4. Data obtained from Dr. Cipto Mangunkusumo General Hospital, Jakarta and Dharmais Cancer Hospital, Jakarta showed an increase in the number of patients with prostate cancer, with the number of patients per year around 70-80 new cases per year.[1] As the second-leading cause of cancer-related deaths, prostate adenocarcinoma is the most commonly diagnosed non-cutaneous malignancy in men. Although early screening and detection has good prognosis, a significant number of men seek treatment with advanced or metastatic disease.[2]

The pathway of androgen hormone activation has an important role in the development and progression of prostate cancer (FIGURE 1).[3] Studies show the presence of intratumoral androgen biosynthesis remains active with increased activity of steroidogenesis enzymes in castrated tumors of the prostate. One of the factors that cause the increase in the activity of intratumoral androgen metabolism is epigenetic changes. In these cases, the process of methylation and change of the

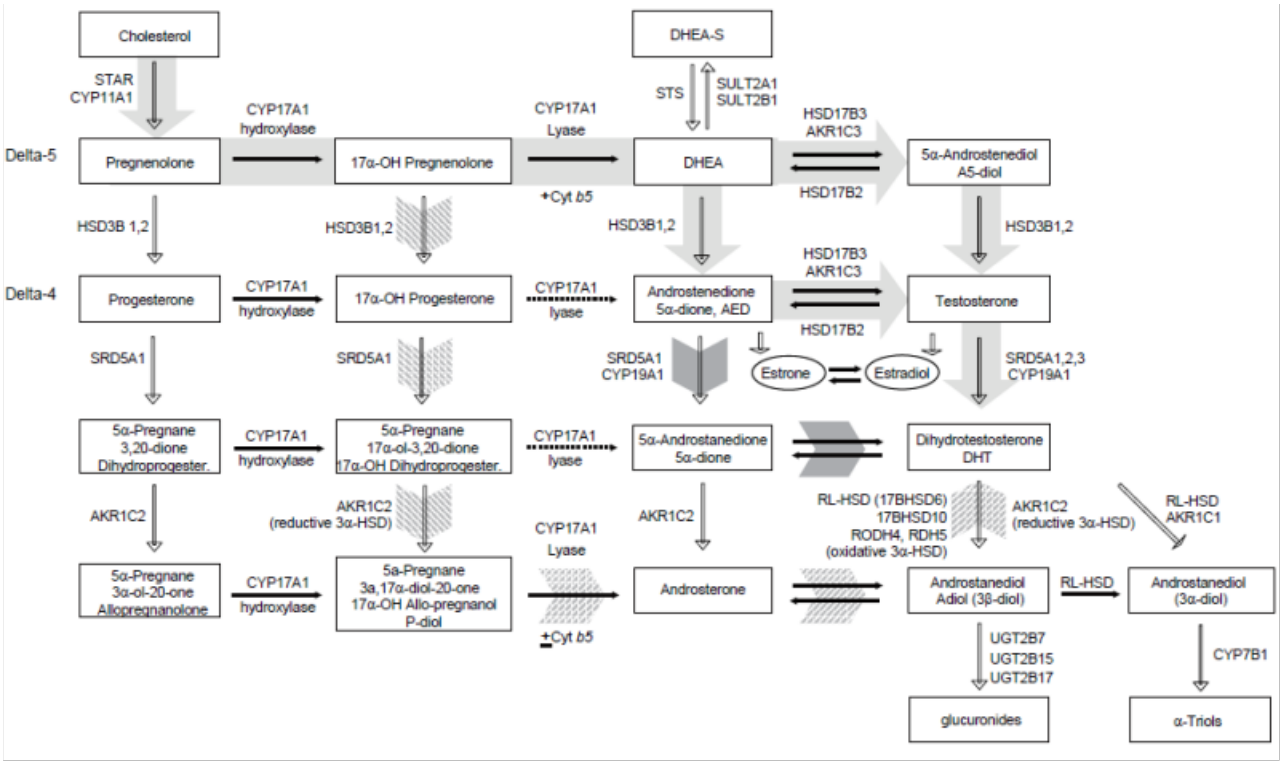


FIGURE 1. Androgen metabolic pathway[4]

DNA structure in prostate cancer cells occur significantly in a number of newly identified steroidogenic enzyme regulatory genes: *CYP17A1, CYP11A1, CYP19A1, HSD3B1, AKR1C2*.[4]

Current strategy in management of prostate cancer mainly involves using hormonal suppression therapy; however, major groups of patients develop castration-resistant prostate cancer (CRPC). A few predictive markers of response to hormonal manipulation have been developed, but due to variation in inter-racial epigenetics, further understanding of epigenetics is needed to develop deliberate therapy on PCa. In this study, we examined each cascade of steroidogenesis in prostate cancer to evaluate our strategy in Indonesia on management of these cases.

## MATERIALS AND METHODS

### Patients

This study was conducted at Urology Division, Department of Surgery, Dr. Sardjito General Hospital, Yogyakarta, Indonesia from September-November 2017. The study was approved by the Medical and Health Research Ethics Committee of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/ Dr. Sardjito General Hospital, Yogyakarta. Samples were taken from 30 paraffin blocks with adenocarcinoma of prostate. The samples were cut and the slices were placed into object glass slides and stained with hematoxylin-eosin (HE). After microscopic reexamination of the samples, we divided the samples into two groups: non-metastatic and metastatic group. Later the samples underwent deparaffinization procedure, and the mRNA expression of *CYP17A1, CYP11A1, CYP19A1*, *HSD3B1, AKR1C2* genes from all of the samples were measured using Real-Time PCR.

### Real-Time PCR

The study lasted for three months in the Anatomical Pathology Laboratory, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta. The course of study was as follows: PCa sample was extracted with Total RNA Kit GeneAll®. Real-Time PCR stage was processed using KAPA SYBR® fast qPCR Kit. Mapping of the samples was programmed within the PCR including the negative control. Mixtures of solution were made with composition as follows: 6.4 µL PCR-grade water, 10 µL KAPA SYBR®, 0.4 µL forward primer (*CYP17A1*/*CYP11A1*/*CYP19A1*/*HSD3B1*/*AKR1C2*), 0.4 µL reverse primer (*CYP17A1*/*CYP11A1*/*CYP19A1*/*HSD3B1*/*AKR1C2*), 0.4 µL deoxyuridine triphosphate (dUTP), 0.4 µL KAPA RT MIX, 2 µL RNA template (TABLE 1). The mixtures were placed into 48 well plates for every primer. Step of PCR and condition of DT lite real-time PCR system were presented in TABLE 2.

TABLE 1. Sequence of the gene primer

| Primer | Forward sequence | Reverse sequence |
|---|---|---|
| GADPH | GCATCCTGG GCTACACTGAG | TCCACCACCCTGTTGCTGTA |
| CYP19A1 | CTGCAGACACTACTACTACA | ATCCGAGTCACTGCTCTCAG |
| CYP11A1 | AGACACTGAGACTCCACCCCA | GACGGCCACTTGTACCAATGT |
| CYP17A1 | AGCTCGTGGCTCTCTTGCTG | CGCGATGTCTAGAGTTGCCA |
| HSD3B1 | TTCCGCCCTCTCTGAGGTACT | GGTCACGAAGTGGCGATTG |
| AKR1C2 | GTAAAGCTCTAGAGGCCGT | CACCCATGGTTCTTCTCGA |

TABLE 2. Step of PCR, condition of DT lite real-time PCR system (DNA-Technology)

| Step of PCR | Temperature | Duration | Cycle |
|---|---|---|---|
| Reverse transcription | 42 °C | 5 min | - |
| Enzyme activation | 95 °C | 3 min | - |
| Denaturation | 95 °C | 1-3 sec | 40 |
| Extension | 60 °C | ≥ 20 sec | 40 |

## RESULTS

The mean mRNA expressions of *CYP11A1, CYP17A1, CYP19A1, HSD3B1*, and *AKR1C2* genes in the metastatic PCa group were 7.08, 10.11, 3.94, 4.84 and 3.58, respectively. In the non-metastatic PCa group, the mean mRNA expressions of *CYP11A1, CYP17A1, CYP19A1, HSD3B1*, and *AKR1C2* genes were 4.62, 9.45, 3.46, 2.68 and 4.92, respectively (TABLE 3). The mean mRNA expressions of the *CYP17A1* gene were the highest expression found in both non-metastatic and metastatic PCa groups.

TABLE 3. mRNA expression of CYP11A1, CYP17A1, CYP19A1, HSD3B1, and AKR1C2 genes

| Genes | PCa | n | Mean |
|---|---|---|---|
| *CYP11A1* | Metastatic | 16 | 7.0813 |
| | Non-Metastatic | 14 | 4.6250 |
| *CYP17A1* | Metastatic | 16 | 10.1113 |
| | Non-Metastatic | 14 | 9.4536 |
| *CYP19A1* | Metastatic | 16 | 3.9425 |
| | Non-Metastatic | 14 | 3.4643 |
| *HSD3B1* | Metastatic | 16 | 4.8469 |
| | Non-Metastatic | 14 | 2.6800 |
| *AKR1C2* | Metastatic | 16 | 3.5800 |
| | Non-Metastatic | 14 | 4.9221 |

There were no significant differences between the mean mRNA expressions of *CYP11A1, CYP17A1, CYP19A1, HSD3B1*, and *AKR1C2* genes in the non-metastatic PCa groups compared to the metastatic PCa groups. This result of groups mean is showed in TABLE 4.

TABLE 4. Mean comparison between groups

| Genes | p | Mean ± SEM* |
|---|---|---|
| *CYP11A1* | 0.206 | 2.45 ± 1.89 |
| *CYP17A1* | 0.772 | 0.66 ± 2.25 |
| *CYP19A1* | 0.740 | 0.48 ± 1.42 |
| *HSD3B1* | 0.095 | 2.16 ± 1.25 |
| *AKR1C2* | 0.474 | -1.34 ± 1.85 |

*SEM: standard error of the mean

## DISCUSSION

Steroidogenesis requires specific hormones and can occur throughout the body. These steroid hormones depend on cholesterol, which is present in all tissues and is necessary for steroid biosynthesis.[5] Steroidogenic acute regulatory protein (STAR) transports cholesterol into the mitochondria in order to convert it to pregnenolone by cholesterol side-chain cleavage enzyme P450scc (CYP11A1).[6] This CYP11A1 activity is essential for steroidogenesis. During this process, pregnenolone is converted either by 3β-hydroxysteroid dehydrogenase (HSD3B) to progesterone or by 17α-hydroxylase (CYP17A1) to 17α-OH-pregnenolone.[7] In this study, both non-metastatic and metastatic groups showed expression of *CYP11A1*, the mean expressions are 4.62 and 7.08, respectively. The mean expressions of *CYP17A1* in non-metastatic and metastatic group are 9.45 and 10.1, respectively.

One important regulator of the balance between androgens and estrogens is aromatase enzyme, encoded by *CYP19A1* gene (*15q21.1*), which is involved in circulating and tissue levels of these hormones in the prostate. Aromatase inhibitors have been found to block the production of estradiol and effective in breast cancer treatment. They pose another possibility for prostate cancer treatment, demonstrating that intraprostatic estradiol may be involved in the disease progression.[8] In this study, both non-metastatic and metastatic groups showed expression of

*CYP19A1*, and the mean expressions were 3.46 and 3.94, respectively.

The *HSD3B* gene family consists of two genes and five pseudogenes, located in chromosome band *1p13.1*. The enzyme 3b-hydroxysteroid dehydrogenase (HSD3B) deactivates dihydrotestosterone (DHT). The type I and type II enzymes are differentially expressed.[4] It was reported that *HSD3B* expression increase in castration-resistant prostate cancer (CRPC), causing increased androstenedione levels, which could generate testosterone.[9] In this study, both non-metastatic and metastatic groups showed expression of *HSD3B1* with the mean expressions were 2.68 and 4.84, respectively. In the previous study, variant allele of *HSD3B1* in Chinese population has been reported by Wu *et. al.*[10] In this population patients who have a variant of *HSDB3B1* gene are more likely to progress to CRPC. This gene might also has a role in the prostate cancer progression in Indonesian population.

*AKR1C2* is a member of the newly emerging Aldo-Keto reductase (AKR) gene family, which metabolize selected steroid hormones, such as progesterone, DHT and androstenedione as well as polyaromatic hydrocarbons. While sharing many similar characteristics such as genomic structure, amino acid and nucleotide sequences, these specific genes catalyze the reduction of selective steroids and are expressed in a variety of human tissues.[11] In this study, both non-metastatic and metastatic groups showed expression of *AKR1C2*, and the mean expression was 4.92 and 3.58, respectively.

## CONCLUSION

In this study, the mRNA expression of steroidogenic enzyme genes was not significantly increased in metastatic compared to the non-metastatic PCa groups. These results suggest that the presence or increased expression of steroidogenic enzyme genesare not clearly associated with metastatic PCa. Steroidogenic enzyme genes can also be seen in prostate cancer not merely in castration-resistant prostate cancer.

The highest mean mRNA expression of steroidogenic enzyme genes was the *CYP17A1* gene, the mean mRNA expression were high both in non-metastatic and metastatic PCa groups. *CYP17A1* gene could be a prognostic factor for prostate cancer in progression to castration-resistant prostate cancer. Further research on protein level with a multicenter study and a larger number of samples are necessary to clarify these results.

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