CHAPTER 4

Discussion and conclusion

Purple rice variant Kum Doisaket in this study has attractively reported its physicochemical and phytochemical compounds (41, 5), leading us to investigate more biological activities of this colored rice. Interestingly, Kum Doisaket rice grain contained high phenolic contents, anthocyanins and antioxidant activity which might relate to their biological activities (5). In rice grains with purple pericarp, the main phenolic contents are anthocyanins cyaniding 3-glucoside and peonidin 3-glucoside (47). In this study, ethanolic extract of purple rice variant Kum Doisaket contained cyaniding 3-glucoside and peonidin 3-glucoside about 2.44 and 0.33 mg/ g extract respectively. Ghasemzadeh *et al.* reported that the phenolic contents in Hashemi rice bran correlated with its antioxidant activity (48). PRE at 1.0 mg of extract showed antioxidant activity equivalent to 56.5 μg vitamin C which related to its phenolic content. The antioxidant phenolic contents in rice could improve the cardiovascular problems (49) and oxidation of LDL cholesterol (50). In addition, phenolic compounds exhibited antimutagens, anticarcinogens and anticancer activity due to their ability to prevent the DNA against the damage and affect cell proliferation (51). Hudson *et al.* reported that active compounds in brown rice decreased cell viability and colony formation of breast and colon cancer cells (52). Furthermore, cyaniding 3-glucoside, the most abundant pigment in purple rice showed anticancer activity through mechanisms of promotion of apoptosis (53), inhibition of cell migration (54) and cell invasion (54, 55). In our previous studies, purple rice extract showed anticancer in both pre and post initiation stages of colon cancer (9, 10). Purple rice extract in various strain showed cytotoxic effect against human hepatocellular carcinoma (HepG2) and prostate cancer cell (LNCaP) (11). Therefore, the active substances in PRE as a result exhibited anticancer activity in several models.

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Although anticancer activities of purple rice have been reported *in vitro* and *in vivo* (9, 10, 11), but the anticancer effects of purple rice in prostate cancer have been less clearly demonstrated. Thus, we decided to determine the effect of PRE on the development of prostatic hyperplasia (BPH). BPH is a common chronic disease in aging men. Although the actual cause of BPH is unknown, there is evidence that testosterone contribute to the development of BPH, due to the high level of testosterone ability to occur the abnormal growth of the prostate (56). In older men, the serum concentration of testosterone decreases while the activities of androgen receptor (AR) are increased due to androgen imbalance. Testosterone plays a key role in prostate growth and development in normal physiological activity. In our preliminary study, castrated rat showed the reduction prostate gland because lacking of testosterone action. Castrated rat then treated with testosterone propionate (3 mg/kg) showed a significant increase prostate size compared to castrated rats and normal rats. The result indicated that high level of testosterone induced the growth of rat prostate gland which is an established model to induce prostatic hyperplasia in rat. Whereas, the normal rat treated with testosterone propionate showed not significant difference of prostate size compared to normal rats. The result suggested that the exogenous testosterone level might be control in rat which remained normal endogenous testosterone. Rolf *el al.* explained that the administration of exogenous testosterone led to downregulation of natural testosterone production by the testes which could control the level of serum testosterone in normal rats (57). Although liver is the main site of androgen inactivation, but the testis has also been showed to catalyze several pathways of testosterone metabolism (58, 59). Therefore, exogenous testosterone could not modulate the prostate growth and development of non-castrated rats. In this study, the BPH experimental rats treated with PRE at 1.0 g/kg body weight showed a significant reduction in prostate weight compared to testosterone-treated alone group ($P<0.05$). The two main classes of drug used for BPH treatment are ndrogen receptor antagonists that block androgen receptor binding and suppress tissue uptake of circulating testosterone such as flutamide and bicalutamide (60) and type II 5α-reductase inhibitor, an enzyme responsible for conversion of testosterone to dihydrotestosterone (DHT) such as finasteride (61). Thus, bicalutamide and finasteride are employed as a positive control. The result of PRE at high dose was consistence with drug control which revealed that finasteride and

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bicalutamide significantly reduced total prostate weight compared to testosterone only treated rats. However, the difference in total prostate weight was not clearly observed, therefore the weight of separated parts of prostate were determined. The anatomical position of the dorsolateral prostate is similar to the peripheral zone of the human prostate, from which carcinoma usually arises. The ventral prostate, the secretory part of prostate, in rat and human does not present to be homologous (62). So the prostates were divided to dorsolateral prostate and ventral prostate and determined the difference of the size. The ventral prostates showed no difference between the testosterone treated alone rats and PRE treated rats. Specifically, the PRE treatment at 0.1 and 1.0 g/kg body weight showed a significant decrease in dorsolateral prostate about 11.43% and 20.0% respectively compared to the rats treated with testosterone alone ($P<0.05$). While the serum level of testosterone in the BPH experimental rats treated with PRE or bicalutamide was similar in each group, finasteride treatment group showed high level of testosterone in serum than other testosterone-treated group. These results demonstrated that PRE attenuated the prostatic enlargement induced by testosterone without the alteration of serum testosterone levels in rats. In histological determination, the epithelium cells of prostate gland in the castrated rat treated with testosterone experienced the proliferation to develop excessive glands and multiple unorganized layers. However, PRE and other drug control showed less progression of hyperplasia but the difference was not clearly observed. These results suggested that PRE trended to reduce the progression of prostatic hyperplasia induced by testosterone. *In vitro* study, the anti-proliferating againt LNCaP (androgen-dependent prostate cell) compared to DU145 (androgen-independent prostate cell) by PRE was determined. The result showed that PRE reduced the proliferation of LNCaP cells but has no effect on DU145 cell. Only LNCaP is prostatic cancer cell which represented to testosterone via androgen receptor, therefore, the growth inhibitory effect of PRE might cause from the modulation of androgen receptor expression or signal transduction.

Androgen receptor has been identified in human prostate including epithelial and stromal cells. It plays an important role in the development and progression of prostate cancer (63). The increasing of AR expression in prostate older rat has been reported. Banerjee *et al.* stated that the expression of AR in the ventral lobe of prostate is decrease but increase in the dorsal and lateral lobes of prostate in Brown Norway rats

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with age (64). As similar with our report, the expression of AR in prostate tissues in TP-induced BPH rats has been elevated compared with the normal rats (65). In addition, the expression of AR in the human BPH tissues is also increased when compared to normal prostate (66). Brolin *et al.* reported that BPH and malignant human prostatic tissue showed the highest proportion of AR-positive cells compared with the normal prostatic tissue (67). Thus, the expression of AR might be a possible contributing factor in BPH and prostate cancer development or progression. On the other hand, AR could be the target for controlling of BPH growth and development. In the present study, the castrated rat treated with testosterone propionate showed increasing the expression of AR compared to castrated control rat. While the high dose of PRE and drug control resulted in a decreasing of AR protein expression in prostate of experimental rats compared to testosterone-treated group. Our findings are consistent with Naiki-Ito (6) who has reported that Ellagic acid (EA) suppressed AR expression resulting in the reduction of progression of prostate carcinogenesis in the transgenic rat for adenocarcinoma of prostate (TRAP) model. EA, a polyphenol found in pomegranate fruit juice, showed a decreasing of AR protein expression leading to induce apoptosis in LNCaP (6). In addition, polyphenol compounds from *Phellinus linteus* also reduced the growth of prostate (68) and the anthocyanins extracted from black soybean could reduce prostate weight in the prostatic hyperplasia-induced rat model (7). Moreover, the anthocyanin treatment can inhibit the growth of prostate cell xenografts in nude mice and also decrease the expression of AR and PSA in prostate cells (8). Especially, cyanidin-3-glucoside (C-3-G) dose dependently inhibited the growth of LNCaP while peonidin-3-O-glucoside (P-3-G) had no effect (69). It also decreased the expression of Cyclin D1and increased the proportion of cells in G0 / G1 (69). Therefore, our study focused in the anthocyanins of color rice. In this study, PRE containing 0.24 % of cyanidin-3-glucoside could reduce the growth of prostate and also could reduce the expression of AR in BPH rats. These results were consistence with our study in LNCaP cells which revealed that PRE significantly suppressed androgen receptor expression leading to reduce the growth of LNCaP cells. The combination of these results suggested that PRE suppressed the growth of prostate gland and prostate cell by suppressing the expression of AR. Therefore, the reduction of AR pathway which might

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modulate the downstream of AR such as prostate-specific antigen (PSA), expression and secretion were determined.

Prostate-specific antigen (PSA) is downstream target proteins of the AR-activated pathway produced by prostate epithelial cells (70) and is the most frequently used marker in the monitoring of prostate cancer. PSA is mainly regulated by androgen signaling pathway (71). In addition, Lee *et al.* reported that androgens induced PSA expression via androgen receptor in LNCaP cells (72). According to AR and prostate growth signaling, therefore, the increasing of PSA level is used as a biomarker of prostate diseases including BPH and prostate cancer. In this study, PRE treatment slightly decreased the expression of PSA detected by western blot, but the quantitative difference was not observed, so the effect of PRE on PSA secretion in culture media of LNCaP was determined. Interestingly, PRE significantly decreased a secretion of PSA in culture media compared to non-treated cancer cells. These findings are consistent with Sun Lee (73) who has reported that Lambertianic acid which could reduce the growth and the expression of AR in LNCaP significantly reduced the cellular and secretory levels of PSA. These results provided evidence to support the role of PRE on the AR function as a growth control.

Normally, testosterone is converted to a more active form, dihydrotestosterone (DHT), by 5α-reductase in prostate cells. Then, DHT binds to androgen receptor to exert its biological functions. An increasing in the expression of 5α-reductase can result in extremely high DHT concentrations in the prostate (74), leading to the accelerating rate of growth in prostate epithelia cell. Therefore, the suppression of the expression of 5α-reductase or inhibit its activity is the strategy for treatment of these diseases. In this study, PRE treatment has no effect on the expression of 5α-reductase mRNA in DU145 cells which showed high level of 5α-reductase and androgen independent growth. Interestingly, Kumar *et al.* studied 5α-reductase inhibitory activity in rat microsomal treated with the crude extracts of Thai plants detected by HPLC. However, red strain of *Oryza sativa* L. was the second most potent 5α-reductase inhibitor of Thai plants (75). Therefore, the effect of PRE on 5α-reductase activity will be further investigated, to define the other mechanism of PRE on prostate carcinogenesis.

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In conclusion, we demonstrated that purple rice extract reduced the growth of testosterone induced rat prostatic hyperplasia and human prostate cancer cell line. These effects were caused from the regulating of AR expression and downstream target pathways of AR signaling. According to these finding the molecular mechanisms and the identified active compound have to be further investigated. Therefore, purple rice might then be further used as a supplement for prostate cancer prevention.

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