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# Low prevalence of Caveolin-1 oncogenic polymorphism G14713A and T29107A among breast cancer patient in Sanglah General Hospital



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## ABSTRACT

**Background:** Currently, breast cancer is one of the most common cancers among women, surpassing only by cervical cancer. Despite the improvement in terms of survivability as well as prognosis, most patients are coming with advanced disease at the time of diagnosis. Unlike early disease, determining the outcome of advanced disease is still challenging. Initial investigation showed that polymorphism within caveolin-1 gene could also contribute in determining risk as well as prognosis. In our initial study, we investigated the prevalence of two oncogenic polymorphism of Caveolin-1: SNP *CAV1* G14713A and T29107A.

**Method:** The samples were obtained by consecutive sampling in Sanglah Hospital from January 2016 to December 2016. The blood samples were collected in EDTA tube and the DNA was isolated using Promega Kyt. Subsequent PCR was conducted to amplify the gene which then sequenced in Genetika Science.

**Result:** We enrolled 43 samples with complete medical records in our analysis. The mean age in our sample was  $49.11 \pm 10.33$  years and mostly stadium III cancer (39.7%) and poorly differentiated. 68% of our sample were luminal types and the rests were HER2 and TNBC with comparable proportion. We only detect one heterozygous sample for G14713A polymorphism in which the patient had Luminal A type but with high Ki67 and poorly differentiated. For T29107A, we detect two heterozygous individuals and one homozygous individual, all with the same characteristics with G14713A.

**Conclusion:** The prevalence of SNP *CAV1* G14713A and T29107A were considerably low in breast cancer population in Bali. However, the characteristic of the samples with the polymorphism suggest that further investigation is needed to confirm their effect in breast cancer morphology.

**Keywords:** Breast cancer, Caveolin-1, Polymorphism, G14713A and T29107A

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## INTRODUCTION

Breast cancer is the second most prevalent cancer among women after cervical cancer with mortality rate reached 522,000 in 2012.<sup>1</sup> The high number of mortality associated with breast cancer is partly caused by the lateness of its detection, causing most patients to seek medical attention with advanced disease (Clinical stage III and IV). Despite global increase in survivability, stage specific 5-year survival for advanced stage breast cancer is considerably low, reaching only 52.4% for stage III and even just 15% for stage IV.<sup>2</sup> The same goes to more malignant subtype such as HER2+ and Triple Negative Breast Cancer (TNBC) which is considered as the most malignant one.<sup>2</sup>

The main cause of poor prognosis and survivability of advanced stage breast cancer is mainly because of the lack of reliable prognostic factor that could predict the behaviour of malignant cell.<sup>3</sup> Current prognostic calculations that rely on clinical and pathological findings are considered to be less reliable because of variety in the molecular pattern

and signalling within the cancer cell.<sup>4</sup> The cancer microenvironment also proved to play a very significant role in determining the behaviour of cancer cell and, thus, the overall prognosis of the disease itself.<sup>5</sup> Cancer microenvironment that composed mainly cancer associated fibroblast (CAF) interacts with cancer cell with various signalling molecule and assist the metabolism of the cancer cell which underlie the Warburg and Reverse Warburg Effect.<sup>6</sup> Warburg effect is the reliance of cancer cell to glycolytic metabolism despite adequate amount of oxygen in its environment while the Reverse Warburg Effect stands for high glycolytic metabolism within the CAF that supply lactate to cancer cell that still maintain its oxidative metabolism.<sup>7,8</sup> These phenomenons had been proved to be a significant predictor of survivability in various types of cancer.

Caveolin-1 is a membrane protein that usually present in the cytoplasmic layer of caveolae. Interestingly, it is down regulated in the occurrence of Warburg/Reverse Warburg effect. Thus,

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it is considered as potential marker for cancer cell behaviour and, hence, the survivability of cancer patients. Various researches have been showed the downregulation of Cav-1 in various kinds of cancer. Most of them showed mixing result. However, its downregulation in breast cancer is always associated with lower survival rate.<sup>9</sup>

Despite numerous researches that evaluate its role in clinical setting, only few research that evaluate the role of genetic variety within *Cav-1* gene that could contribute to its down regulation. Two studies in Taiwan revealed that there were indeed several types of single nucleotide polymorphism in *Cav-1* gene.<sup>10,11</sup> Two of them were proved to be a moderate risk factor for leukaemia in children and breast cancer. However, there is no such research that ever conducted in Indonesia. Considering the different race between Taiwanese and Indonesian population and poor cancer genetic data in Indonesia, it is necessary to evaluate this SNP in order to reveal its role in Indonesian population.

## METHODS

A cross-sectional research was conducted in Sanglah General Hospital from January to December 2016. The subjects were enrolled consecutively and asked for informed consent before participation. Patients baseline data were collected from medical record and tabulated by excel. Three ml of blood sample was collected from each subjects and stored in EDTA tube in -20°C. The DNA was isolated by using PROMEGA Blood DNA Isolation Kit at Biochemistry Department Faculty of Medicine Udayana University. The *Cav-1* gene were isolated and amplified by Polymerase Chain Reaction using two kinds of primers: 5'-CCTTCCAGTAAGCAAGCTGT-3' and 5'-CCTCTCAATCTTGCCATAGT-3 for G14713A and 5'-GCCTGAATTGCAATCCTGTG-3' and 5'-ACGGTGTGAACACGGACATT-3 for T29107A. The PCR products were visualized using Agarose Gel Electrophoresis and isolated. The purified gene then sequenced at Genetika Science for polymorphism detection.

## RESULTS

### Baseline Characteristics

The baseline characteristics of the subjects are presented in the table below (Table 1). The mean age of the subjects was 49.11±10.33 years which range from 28 to 78 years. The proportion of pre-menopausal and post-menopausal was almost

even with more than half of the subjects had cancer on the right breast (65.1%). Most of our subject came with stage III breast cancer (39.7%) with high histological grade (38.1%) while early stage breast cancer only consist minimal proportion. From subtypes, the majority of our subjects had Luminal B followed by HER2.

### The Prevalence of *CAV-1* Polymorphism SNP G14713A and T29107A

The prevalence of *CAV-1* SNP G14713A and T29107A in our subjects population were very rare. There was only one subject that has G14713A with heterozygote genotype while there were three subjects that have T29107A in which one is homozygote and the others were heterozygous. The one with G14713A had grade II and Luminal B breast cancer. Those who had heterozygous T29107A genotype also had the same cancer morphology while the one who had homozygote phenotype stadium IV and Luminal B with grade III breast cancer.

**Table 1** Subjects baseline characteristics

	VARIABEL
Umur	Mean: 49.11±10.33 Tahun Range: 28-78 Tahun
Status Menstruasi	Premenopause : 20 (46.5%) Post Menopause : 23 (53.5%)
Lokasi Tumor	Mammae Kanan : 28 (65.1%) Mammae Kiri : 15 (34.8%)
Kuadran	Central : 23 (36.5%) Upper Outer : 8 (12.7%) Kuadran Lain : 12 (19.2%)
Stadium Klinis	I : 1 (1.6%) II : 3 (9.6%) III : 25 (39.7%) IV : 14 (22.2%)
Grade Histologis	I : 3 (4.8%) II : 14 (17.5%) III : 26 (38.1%)
Reseptor	Reseptor Estrogen Positif : 20 (46.5.8%) Negatif: 23 (53.5%) Reseptor Progesteron Positif : 18 (41.8%) Negatif: 25 (58.13%) HER2 Positif : 24 (55.8%) Negatif: 19 (44.18%)
Subtipe	Luminal A : 9 (20.93%) Luminal B : 18 (41.8%) HER2 : 10 (23.25%) Triple Negative: 6 (13.95%)

## DISCUSSION

Breast cancer is the second most common cancer in women and also has the second highest mortality rate. Despite increased in overall 5-year survival rate, advanced cancer still posed a challenge to oncologist because of difficulties in determining the prognosis. It mainly cause by diversity in cancer cell molecular properties which make it difficult to determine when to move directly to more aggressive treatment or starting with the conservative one.<sup>1,2</sup>

Cancer metabolism has been proved to play a significant role in altering the malignant properties of cancer cell.<sup>7,8</sup> Cancer often relies so much on glycolytic pathway even in adequate concentration of oxygen, the metabolism model that latter defined as Warburg Effect. The product of glycolysis, pyruvate and its reduction form, lactate, is a perfect source of carbon backbone used to synthesize other macromolecule such as various amino acid and fatty acid.<sup>8</sup> These are essential to support highly mitotic cell for protein and membrane construction. The other means of metabolism that just recently discover is Reverse Warburg Effect. The principle is still the same but glycolytic process occurs in CAF instead of cancer cell. Cancer cell produce high amount of free radicals that induce mitochondrial degradation in CAF which made it rely on glycolysis and lactic acid fermentation to synthesize ATP.<sup>8</sup> The by product, lactate, then exported out of cell by MCT-1 and taken up by cancer cell to either directed to energy production or synthesize of macromolecule.

The importance of metabolic change and microenvironment in cancer is immense since it is supported almost all cancer cell characteristics such as proliferation, metastasis, angiogenesis, immune-evasion and modulation, and resistance to apoptosis.<sup>12</sup> Several researches proved that culturing cancer cell with fibrous tissue increase the proliferation rate and invasive capabilities of cancer cell.<sup>13,14</sup> Furthermore, interruption of cancer metabolism by 3-bromopyruvate proved to be effective in treating hepatocellular carcinoma in vivo.<sup>15</sup>

Free radicals are not just altering the metabolism of cancer cell and CAF, but also induce down regulation of Cav-1.<sup>8</sup> Current researches proved that Cav-1 is not just structural protein, but may also alter signalling pathway within cancer cell or CAF.<sup>12</sup> Down regulation of Cav-1 has been proved to associate with increased cell proliferation, increased HIF-1 $\alpha$  activation, and increased TGF- $\beta$ , IL-8 and IL-10 production. It also associated with genomic instability which increases mutational rate and the chance of beneficial mutation for cancer cell. These effects could be the result of metabolic alteration

that underlying Cav-1 down regulation but also might because the signalling properties of Cav-1 itself which is still unknown.<sup>12</sup>

Because of its importance, SNP that alter the performance or the rate of synthesis of Cav-1 could also play a role in carcinogenesis or cancer progression. Two previous researches, both conducted in Taiwan proved that two SNPs in *Cav-1* gene were associated with slight increase in cancer risk.<sup>10,11</sup> Research conducted by Liu et.al proved that SNP G14713A and T29107A were related with increased risk of leukaemia in children.<sup>10</sup> Meanwhile, in separate study, they also found that the same SNPs were associated with increased risk of breast cancer in Taiwanese population with OR 1.50 (95% CI=1.14-2.12).<sup>11</sup> However, the effect of the SNP toward the function or rate of synthesis of Cav-1 is still unknown.

However, our study proved that oncogenic polymorphism of *Cav-1* gene may not play a significant role in Balinese population. It appeared that both SNP were very rare in Bali and did not contribute in the carcinogenesis of breast cancer. However, our previous research (unpublished) proved that Cav-1 was indeed often down regulated in breast cancer. Thus, the role of Cav-1 in breast cancer progression in Balinese population seems to cause by its down regulation by means of increased free radicals production by cancer cell. However, the low number of sample in this research might cause a bias and deviate our finding with the general population

## CONCLUSION

Our finding suggests that SNP G14713A and T29107A of Cav-1 gene may not play a significant role in breast cancer in Balinese population. However, due to unknown status of cancer genetics in Balinese and Indonesian population, research on other potential SNP should be conducted in order to fully characterize the SNP pattern of cancer in Indonesia particularly breast cancer.

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