Cervical Cancer

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Cervical cancer is one of the most common malignancies in women worldwide and its management remains challenging and complex. As Cytochrome4Z1 (CYP4Z1) is overexpressed in many tumours, its expression in cervical cancer is unknown. Therefore, the present study aimed to evaluate CYP4Z1 expression in cervical cancers. Methods: CYP4Z1 expression was immunohistochemically assessed in 100 cases of cervical cancers along with ten normal cervix tissues, and the enzyme's relationship to several clinicopathological features and survival was explored. Results: CYP4Z1 was strongly expressed in 55% of cervical cancer patients. Normal cervix samples were negative for CYP4Z1 expression. Importantly, this expression was significantly found in patients with the late stage of the disease, lymph node metastasis, and high tumour invasion ($p < 0.05$). Interestingly, CYP4Z1 expression was significantly correlated with shorter survival times of cervical cancer patients. Univariate analysis showed that CYP4Z1 expression, tumour stage, lymph node metastasis, and tumour invasion were significantly correlated with patient survival ($p < 0.05$). The multivariate analysis revealed that only CYP4Z1 expression and tumour stage were significantly correlated with patient survival ($p < 0.05$). Conclusions: CYP4Z1 expression is associated with cervical cancer patients' survival and may serve as an independent predictor of poor prognosis in cervical cancer patients.

cancer cervical cancer cytochrome P450 cytochrome4z1 immunohistochemistry

1. Introduction

Cervical cancer ranks as the fourth most prevalent cause of cancer mortality and morbidity in women worldwide [1][2]. According to the International Agency for Research on Cancer report GLOBOCAN 2018, the annual cervical cancer burden reaches 570,000 new cases and about 311,000 deaths from cervical cancers globally [3]. This type of tumour is mainly triggered and developed by persistent infection by human papilloma virus (HPV). This is a sexually transmitted virus that is classified into high-risk and low-risk types. In particular, the most common aggressive types of virus, causing approximately 70% of cervical cancers, are HPV 16 and 18 [4][5]. In recent decades, the burden of HPV in cervical cancer has decreased because of the effective implementation of cervical screening and HPV vaccination programmes and improvements in therapeutic strategies. However, cervical cancer mortality remains high in some regions of the world, particularly in developing countries, due to a lack of screening and vaccination programmes [2][5][6]. Based on these facts, there should be an urgency to accelerate the development of novel biomarkers and targeted therapies to better manage cervical cancer.

The role and significance of cytochrome P450s (CYPs) in the carcinogenic process have contributed to the development of cancer therapies based on the expression and metabolic pathways of CYPs [7]. Of specific interest

is the aberrant expression of CYP4Z1 in breast cancer. CYP4Z1 selective expression in breast cancer has inspired researchers to characterise its expression in other cancer types and question its effect on cancer development [8][9][10][11]. Clinical studies exploring the expression profile of orphan CYP4Z1 and its association with clinicopathological parameters, albeit limited, demonstrate an interesting trend. Several studies reported differential expression of CYP4Z1 in cancers of the breast, ovary, and prostate [8][11][12][13]. Recently, we characterised CYP4Z1 expression in bladder [14]. Importantly, its expression is associated with poor patient prognosis and has been suggested as a biomarker for cancers of the ovary and prostate [12][13]. Moreover, the expression of CYP4Z1 was able to differentiate between benign, primary, and malignant breast and ovarian tumours [15]. Of interest is the finding that the cell surface of breast cancer has shown an abnormal translocation of CYP4Z1 expression compared to nothing displayed on the surface of normal breast cells [16]. This aberrant cell surface localisation enhances the development of CYP4Z1 autoantibodies in breast cancer patients' sera and is proposed as a diagnostic biomarker for breast cancer [17]. Therefore, CYP4Z1 may show potential as a biomarker and in the development of targeted therapies selectively directed at the tissues in which it is expressed.

The high expression and poor prognosis association of CYP4Z1 prompted both in vitro and in vivo studies to unravel the enzyme's contribution to tumour development. CYP4Z1 was conditionally overexpressed in breast cancer cells when treated with glucocorticoids and progesterone. Interestingly, treatment of these breast cancer cells with a steroid-receptor blocker, mifepristone, reduced CYP4Z1 conditional overexpression [18]. Importantly, CYP4Z1 expression significantly enhanced tumour growth, angiogenesis, and spread of cancer cells in both in vitro and in vivo models. These effects were biochemically accompanied by a reduction in fatty-acid levels, particularly lauric and myristic acids, and an increase in 20-hydroxyeicosatetraenoic acid (20-HETE) levels [19]. Such biochemical hydroxylase and epoxygenase activities of CYP4Z1 were reported by several studies [20][21][22][23]. CYP4Z1 was capable of metabolising lauric and myristic acids to monohydroxylated products and arachidonic acid to 20-HETE [21]. However, a recent report has identified CYP4Z1 to exclusively metabolise arachidonic acid to14,15-epoxyeicosatrienoic acid (14,15-EET) rather than 20-HETE [22]. Overall, CYP4Z1 biochemical activities towards arachidonic acid metabolism to either 20-HETE or 14,15-EET have been proposed as a causative mechanism contributing to tumour development [22][23]. This may provide a possible molecular pathway for CYP4Z1-driven tumour progression. However, the CYP4Z1 enzyme's association with cancer development remains the focus of current research.

2. Current Insight on Cervical Cancer

Cervical cancer has become a major health concern due to the rise in mortality and morbidity around the world [2][3]. This rise, particularly in developing countries, is attributed to poor screening and vaccination programmes against HPV [3][5][6]. This disease is considered more aggressive and has a relatively worse prognosis [3][4]. Owing to the lack of targeted therapies, the clinical management of cervical cancer is challenging and remains difficult. As a result, there is an important need to find novel biomarkers and drug targets that can improve cervical cancer clinical management. While considered an interesting area of study, new and existing research opportunities emerge to unveil novel aspects of CYP4Z1 in cancer development and therapy. Our recent initial screening has

identified overexpression of CYP4Z1 in a small number of tumour samples for each type of human tumour including cervical cancer [11]. Therefore, the implications of this observation have been taken to fully characterise the CYP4Z1 expression in a large cohort of cervical cancers.

As the current study was the first investigating the CYP4Z1 expression in cervical cancers, we found that 55% of the tumours expressed CYP4Z1, where the expression in each tumour sample was specifically confined to tumour cells. Normal cervix tissues showed no CYP4Z1 expression at all. The high frequency of CYP4Z1 expression in cervical cancer is consistent with the findings of our earlier initial screening [11]. Moreover, our results agree with CYP4Z1 transcription profiling in cervical cancers shown by the Human Protein Atlas. Low to high CYP4Z1 mRNA levels were identified in cervical cancers compared to normal cervix tissues [24]. Importantly, this CYP4Z1 differential expression between normal tissues and tumour tissues was significantly observed in many studies. In these studies, CYP4Z1 was expressed at much higher levels in cancers of the breast, ovary, and prostate than in their corresponding normal tissues [8][10][11]. Moreover, we recently identified a similar fashion of CYP4Z1 differential expression in bladder [14] and colon cancers (data not published).

The role of the CYP4Z1 enzyme as a prognostic marker in cervical cancer was assessed in this study. This was the first study indicating a significant correlation between CYP4Z1 expression and cervical cancer patients' survival. CYP4Z1 expression was associated with poor survival rate and identified as an independent factor for poor prognosis in cervical cancer patients, along with tumour stage. These findings are in agreement with previous studies identifying CYP4Z1 as a prognostic marker for ovarian and prostate cancers [12][13]. Further significant associations were found between CYP4Z1 expression and tumour invasion and lymph node metastasis. These findings demonstrate the possible role of the CYP4Z1 enzyme in the progression and malignancy of cervical cancer.

As few functional studies have interrogated the role of CYP4Z1 in cancer development particularly breast cancer [16][19][20][25], mechanisms behind functions of CYP4Z1 in cervical cancer progression are still unknown. By using in vitro and in vivo models, CYP4Z1 overexpression was found to promote breast cancer-cell invasion, migration, proliferation, and tumour angiogenesis [16][19][20]. This was particularly triggered by activation of ERK1/2 and PI3K/Akt signalling pathways through increased expression of vascular endothelial growth factor-A (VEGF-A) and decreased production of the tissue inhibitor of metalloproteinase-2. It is important to note that all of these changes were biochemically associated with increased production of 20-HETE and 14,15-EET [19][22]. Such CYP4Z1 enzymatic activity of metabolising arachidonic acid to either 20-HETE or 14,15-EET was reported by many studies [21][22]. Importantly, the 20-HETE was shown in many studies to work in conjugation with VEGF, enhancing tumour angiogenesis, growth, and metastasis [26][27]. Beside VEGF, 20-HETE was shown to be involved in activation of the PI3K/Akt- and mitogen-activated protein kinase (MAPK) pathways necessary for proliferation and survival of cancer cells [28]. Regarding 14,15-EET, it was reported that 14,15-EET promoted tumour angiogenesis by stimulating tyrosine-protein kinase (Src) and activated transcription-3 (STAT-3)-dependent production of VEGF [29]. Moreover, 14,15-EET was found to partly regulate the pro-tumourigenic pathways of PI3K/Akt, MAPK, and VEGF [22][30][31]. The activation of these signalling pathways in HPV-induced cervical cancers wwas reported by numerous studies [32][33][34]. Further analysis of the mechanistic role of CYP4Z1 in the tumourigenesis process showed that synergic

expression of pseudogene CYP4Z2P and CYP4Z1-3'UTRs enhanced tumour neovascularization in breast cancer partly through activating pathways of PI3K/Akt and ERK1/2 [20]. Moreover, overexpression of CYP4Z1 and/or CYP4Z2P in breast cancer cells may promote transcriptional activity of oestrogen receptors, stemness, and tamoxifen resistance [25]. Taken as a whole, these findings may provide a plausible mechanism for CYP4Z1-driven tumour development. However, the link between the CYP4Z1 enzyme and cervical cancer development remains elusive.

Knowledge in the field of the CYP4Z1 enzyme's substrate recognition and catalytic properties is now quite valuable in the design and development of more selective cancer therapies. A limited number of reports explored the substrate-binding mode of CYP4Z1 [35][36][37][38]. Several key amino acid residues have been identified for substrate binding of CYP4Z1 including Arg487, Asn381, Ser383, Ser222, Ser113, and Asn381 [35][36]. Recently, luciferin benzyl ether was identified as the best luminogenic substrate for CYP4Z1 using the permeabilised cells of fission yeast expressing CYP4Z1 [36]. These recent advances in determining the CYP4Z1 enzyme's substrate recognition have led to the development of selective inhibitors for CYP4Z1. The first inhibitor identified was 1-benzylimidazole, which showed efficient blocking ability for production of 14, 15-EET in CYP4Z1 positive tumour cells relative to a poor inhibitory profile against other CYPs [38]. Interestingly, a new highly potent inhibitor (Compound 9) for CYP4Z1 was developed using systematic virtual screening. This novel inhibitor showed selective binding and high nanomolar affinity to CYP4Z1 [37]. These latest advances may accelerate the development of CYP4Z1 targeted therapies.

The future of cancer therapy relies mainly on the use of biomarkers that guide clinicians in each step of cancer management. For this purpose, there is an increasing interest in discovery and development of novel biomarkers that help in cancer diagnosis, prognosis, and monitoring treatment response [39]. For cancer diagnosis, biomarkers can be used for cancer risk assessment, early detection of cancer, and accurate staging of disease. For instance, analysis of differentially expressed protein in discrimination between normal and cancer tissues [40]. Regarding cancer prognosis, biomarkers help in estimating the course of cancer disease and therefore the most suitable management strategy. Moreover, biomarkers can also be used for predicting the response of patients to various treatment strategies [41]. In this case, for example, the biomarker expression levels generally reflected the extent of cancer burden, therefore, high levels of biomarker mostly indicated poor prognosis and sometimes the opposite. As it reflected the cancer burden, biomarkers could also be used in the cancer staging system [40]. Overall, the discovery of novel biomarkers probably CYP4Z1 may hold promise for cervical cancer management.

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