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A Hypothetical Mechanism of how HPV E6 and E7 Infections inactivate BRCA1 Function Resulting in Cervical Cancer

Research Article

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Abstract

Cervical cancer is one of the most common female cancers, with Human Papilloma Virus (HPV) infection being the major risk factor. Although regular screening for cervical cancer has been associated with reduced incidence of the disease, it is still critical to investigate how the cancer develops, so that we can improve treatment options. Previously, HPV E6 and E7 oncoproteins were shown to interact with BRCA1 to inactivate its functions but how this may initiate cervical cancer was unknown. Ubc9, p16 INK4 expression is strongly upregulated and ER-α is declined in cervical lesions. We and others observed a similar scenario in triple negative breast cancer (TNBC) with BRCA1 mutation or dysfunction where loss of binding to its downstream target Ubc9 results in ER-α repression and high p16INK4 expression. We therefore hypothesize that HPV E6 E7 oncoproteins by tethering BRCA1 inactivates its function causing loss of binding to Ubc9 resulting in its accumulation, ER-α downregulation, high p16INK4 levels resulting in cervical cancer. In summary, we have proposed a novel hypothetical molecular mechanism as to how HPV oncogenes interfere with BRCA1 function contributing to the development of cervical cancer.If confirmed future work will help in using Ubc9 and its downstream targets as potential biomarkers for early diagnosis and/or monitoring the progression of HPV oncogenic infections and for designing drugs that target Ubc9 expression to combat these aggressive cancers.

Keywords: Cervical Cancer; HPV E6 E7; BRCA1; Ubc9; ER-α; p16 INK4; etc.

Abbreviations: HPV: Human papillomavirus, TNBC: Triple Negative Breast Cancer; ER: Estrogen Receptor; AA: African American;Ubc9: SUMO E2- conjugating enzyme 9.

Introduction

HPV and Cervical Cancer

Cervical cancer is one of the most common cancers in women, despite a decline in both incidence and mortality rates over the past 43 years (1975-2018), with 14,100 estimated new cases and 4,290 estimated deaths in 2022 [1]. Hispanic women have a higher incidence of cervical cancer than non-Hispanic Black and White women, while non-Hispanic Black women have a higher mortality rate than Hispanic and non-Hispanic White women [1]. Regular screening for cervical cancer has strongly reduced cervical cancer incidence, as it enables us to identify and eliminate early neoplasms. Human Papilloma Virus (HPV) is the most common risk factor for cervical cancer, with HPV DNA found in 99% of invasive cervical cancers worldwide [2, 3]. Most cervical cancers involve the interactions between HPV oncogenes E6 and E7 with tumor suppressor genes like p53 and pRB promoting their degradation [4]. Focusing on these interactions, we have proposed a novel mechanism by which E6 and E7 oncogenes cause cervical cancer via inactivation of BRCA1 function. BRCA1 and BRCA2 are tumor suppressor genes essential for the repair of damaged DNA,and mutations in these genes increase the risk of devel-

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oping several types of cancers,most notably breast and ovarian cancers. However, studies have shown that BRCA1 and BRCA2 gene mutations can also increase a woman's risk of developing cervical, uterine, pancreatic, colon, stomach, esophageal, and liver cancers [2].

BRCA1 and Cervical Cancers

BRCA1 and its isoforms function as growth / tumor suppressors in TNBC, ovarian and prostate cancer cells and mice xenografts [5]. In normal cells, BRCA1/1a/1b proteins bind to a downstream target Ubc9 to facilitate nuclear localization of BRCA1, resulting in the activation of ER-α BRCA1 mutations/dysfunction disrupt this normal process leading to loss of binding to Ubc9, resulting in high levels of Ubc9 which enters the nucleus and suppresses ER- α, causing TNBC [6, 7].

A similar scenario is seen in cervical cancers where Ubc9 is strongly upregulated, increase in p16INK4 expression and ER-α to be strongly repressed in cervical cancer lesions [8, 9]. HPV E6 and E7, ER- α oncoproteins have been shown to inactivate BRCA1 function [8] in cell lines (siHA, Caski and HeLa).An inverse relationship is observed between ER- α and p16 INK4 in cervical cancer tissue, suggesting that HPV infection can cause loss of ER- α expression and increase in P16 INK4 as the cells become cancerous, p16 INK4 is a marker for HPV linked cancers [9]. Based on these observations we put forth a novel molecular mechanism of inactivation of BRCA1 by HPV E6/E7 infection results in loss of binding to Ubc9 which at high levels, represses ER- α and increases p16 INK4 activity, apoptosis inhibition by inactive cytoplasmic BRCA1 resulting in cervical cancer.

HPV oncoproteins are known to interact with p53 to upregulate Ubc9. Specifically, the E6 oncoprotein can form a complex with p53 and a ubiquitination enzyme E6-AP [10]. By binding and degrading p53, cell cycle checkpoints can be bypassed, and tumor cell progression can be enabled. The E7 oncoprotein on the other hand binds to the Rb domain that is responsible for tumor suppression. Normally, Rb binds to E2F-family transcription factors to regulate the cell cycle. When the E7 oncoprotein binds to Rb to disrupt its interaction with E2F, E2F factors are released in their active forms, and the cell cycle can be constitutively active, thus allowing for tumor formation. During cancer formation and progression, oncoproteins HPV E6 and E7 can concurrently bind to cell cycle mediators p53 and Rb, as well as BRCA1 [8]. In binding to BRCA1, HPV E6 and E7 bind to amino acids 67-100 and 1532-1749 in the C-terminal domain of BRCA1. As opposed to the degradation seen in p53 and Rb upon binding to the oncoproteins, BRCA1 is not degraded but loses its function upon binding to HPV E6 and E7 [8]. Previously a new BRCA1-Ubc9 nuclear trafficking pathway was identified and BRCAness was found to perturb this balance resulting in TNBC [11].

HPV- positive Cervical Cancers and Ubc9

Sumoylation, the post-translational modification process of adding a small ubiquitin-like modifier or SUMO moiety to proteins, is mediated by the E2-conjugating enzyme Ubc9 [12]. As such, Ubc9 levels are implicated in an array of cellular functions by interacting with and regulating cell cycle proteins and tumor suppressors [13, 14]. A proposed mechanism of Ubc9-related tumorigenesis has been documented in the literature, with high levels of Ubc9 implicated in cancers [15]. Though not exhaustive, a few examples of Ubc9's involvement in cancer include its role in breast cancer metastasis and tumor cell invasion, as well as its involvement in cervical cancer tumor progression [16, 17]. Experiments carried out by Mattoscio et al. demonstrated that HPV-positive cervical cancer lesions displayed higher Ubc9 levels compared to HPV-negative head and neck cancer lesions; results also demonstrated an upregulation of Ubc9 correlating with cervical cancer lesion progression [18]. It was speculated that the upregulation of Ubc9 was promoted by the E6 and E7 oncoproteins via p53. This upregulation prevents cell apoptosis. By itself, Ubc9 reduces apoptosis, while the E6 and E7-induced transformation leads to apoptosis resistance [15, 18]. Reduction of apoptosis in cells would lead to a higher likelihood of tumorigenesis. This implies that HPV E6 and E7 oncoproteins inhibit Ubc9 degradation, thus allowing Ubc9 accumulation as well as apoptosis-resistance in the cell. Thus, Ubc9 detection can be used as a biomarker in diagnosing and/or monitoring the progression of HPV oncogenic infections [18].

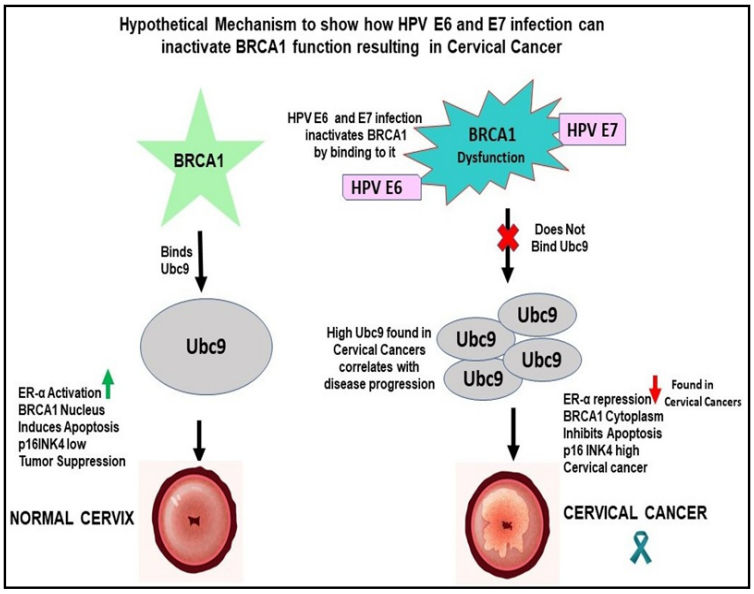
P16 INK4 loss rescues BRCA1 Function:

BRCA1 is a known tumor suppressor that is involved in several pathways of DNA repair [19, 20]. Cao et al., demonstrated that a lack of BRCA1 caused premature senescence in cultured cells as well as tumorigenesis in mice [21]. The same study found that this malignant transformation of cells was mediated by p53 [21]. Interestingly, Schuyer and Burns found that BRCA1-related breast cancers contained a higher amount of p53 mutations relative to sporadic cancers [22].

p16INK4a (p16) is a cyclin-dependent kinase inhibitor that limits the progression of the cell cycle from G1 to S [23]. This kinase inhibitor has been extensively studied and reported in the literature as a biomarker for cervical cancer [24-26]. Khleif et al.,[23] found an inverse relationship between p16INK4a and retinoblastoma tumor suppressor protein (RB) in which mutated, deleted, or inactivated RB led to higher expression of p16INK4a protein [23]. As previously mentioned, HPV E7 binds and inactivates RB,causing the release of E2F factors. Interestingly, Farzanehpour et al. demonstrated a direct relationship between the overexpression of p16INK4a and the severity of cervical cancer [27]. Moreover, Lau et al [28] found that cells transfected with p16INK4a small interfering RNA (siRNA) had a much higher rate of apoptosis when exposed to ultraviolet irradiation and cisplatin compared to the siRNA control counterpart, thus suggesting that p16INK4a is implicated in the cellular apoptosis response [28]. Lastly, BRCA1 also interacts with Nrf2 to regulate cell survival [29].BRCA1 loss or inactivation has been implicated as a cause of tumorigenesis in the literature [30, 31]. Its role in tumorigenesis lies in its regulation of DNA damage checkpoints. Loss or inactivation of BRCA1 results in premature senescence and apoptosis-resistant cells [31]. Scott et al. [32] further demonstrated that disruption of BRCA1 function could lead to premature senescence in mammary epithelial cells [32]. However, the same study also found that loss of p16INK4 rescued BRCA1 from loss of function by reducing senescence in the mammary epithelial cells. This finding suggests that p16INK4 is involved in DNA damage repair and cell senescence and could be a potential downstream target in the BRCA1 pathway.

Clinical implications: HPV oncogenes [E6, E7] are known to

Figure 1. Shows a hypothetical molecular mechanism as to how HPV E6 and E7oncoproteins by tethering BRCA1 inactivates its function resulting in loss of binding to Ubc9 and its localization to the cytoplasm. Ubc9 binds and represses ER- α activity, p16INK4 levels are high due to inactivation of BRCA1 resulting in cervical cancer.



play a vital role in malignant transformation. E6 oncoproteins target cellular tumor suppressor protein p53 for inactivation and degradation [33]. Likewise, E7 oncoproteins bind to RB, disrupting its interaction with E2F, leading to uncontrolled tumor replication [33]. The interactions between E6 and p53 as well as E7 and RB impair Ubc9 degradation and cause accumulation of it in cells and tissues which increases host cell resistance to apoptosis [34]. These interactions can be targeted for therapeutic interventions to regain function of the tumor suppressor genes [33]. Also, disrupting the interaction between BRCA1 and the E6/E7 oncoproteins is another viable way that can be explored for treating cervical cancers.

Conclusion

In conclusion several studies have shown an increased risk of TNBC and cervical cancers in carriers of BRCA1 mutation or dysfunction caused by HPV infection [2]. A molecular pathway as to how BRCA1 mutation/dysfunction results in TNBC was shown [6, 11] whether a similar mechanism is involved in triggering cervical cancer following HPV infection needs to be investigated. Based on this, we hypothesize that HPV oncogenes interfere with BRCA1 function leading to cervical cancer.The next step from here is to continue the investigation into the proposed molecular mechanism involving HPV oncogenes disrupting BRCA1 functions and study how Ubc9, p16INK4 and ER-α can be used as biomarkers for early detection or as potential therapeutic targets for BRCA1-associated cervical cancers which can rescue BRCA1 function.

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