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Short Communication

Snapshot of Clinical Implications of p16 Overexpression in Carcinogenesis

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ABSTRACT

Molecular markers are needed to decide the treatment plans of certain cancer types when the histological and other clinical diagnoses are not sufficient to decide the tumor nodular metastasis (TNM) stage. The ubiquitous p16 gene is one of them gained popularity by fulfilling criteria to be a useful biomarker. Over expression of p16, to compensate the inactivity of another two tumor suppressor genes (TSG)s, pRb and p53 due to the integration of E7 and E6 high risk Human Papilloma viral oncoprotein respectively into the host keratinocytes is useful to consider the clinical impact of p16 biomarker in carcinogenesis. The p16 immunohistochemistry helps the diagnosis as well as prognosis of cervical and oropharyngeal squamous cell carcinoma, though there are ambiguities in the cutoff values of p16 positivity. There is also a re-emerging interest on clinical impact of p16 positivity in lung, breast, and colorectal cancer types. High risk HPV genotypes have been already established as the aetiological agents of cervical, other rare ano-genital and oropharyngeal (especially tonsils and base of the tongue) cancers. The HPV associated subset of head and neck cancers demonstrate a unique tumor biology, when compared with HPV non associated ones thus, most effective treatment planning including counselling is much needed to maximize the overall survival of HPV associated cancer patients, in the era of personalized precision medicine. In the shed of light, this communication glimpses on clinical implications of p16 overexpression in carcinogenesis not limiting to cervical and a subset of head and neck carcinomas (HNSCC).

Communication

Carcinogenesis is a complicated process initiated by activation of critical mutations due to genetic and epigenetic changes via multiple pathways [1]. These genetic and epigenetic changes could occur in proto-oncogenes (which convert them into oncogenes) tumor suppressor and DNA repair genes undergoing cycles of evolutionary clonal selection to transform normal cells into malignant cells [2]. Disruption of harmonious balance between cell proliferation and programmed cell death termed apoptosis is the consequence of aberrant expression of oncogenes and inactivation of tumor suppressor genes (TSGs) in any cancer type [3]. There are well known tumor suppressor genes which have become the ideal candidates for mechanistic studies. Among them, tumor suppressor, p16Ink4a is the principal member of the Ink4 family of cyclin-dependent kinase (CDK) inhibitors involved in the retinoblastoma protein (Rb) pathway, in the regulation of cell cycle by inhibiting the progression of G1 to S phase of cell cycle [3]. However, the full competency of this putative biomarker is yet to be unveiled [4].

Antiproliferative p16 is ubiquitous and not limited to a particular cancer cell line or specific cancer type [5]. Thus, *in vitro* experiments have demonstrated a high frequency of p16 deletion in melanoma, lung, pancreas, mesothelioma, bladder, head and neck, breast, acute lymphocytic leukemia, brain, osteosarcoma, ovarian and renal cancer cell lines which were not infected with Human Papilloma Virus (HPV) [6-8]. In contrast, upregulation of p16 is observed through a negative feedback loop after functional inactivation of pRb and ubiquitination of p53 by oncogenic E7 and E6 viral gene products respectively [9]. In this scenario, anti-proliferative p16 gene product seems to elevate for

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compensation of non-functional state of foremost tumor suppressor pRb and p53 genes [9-11]. Thus, over expression of p16 has become the bio marker of choice to identify transcriptionally active, hence high-risk HPV, by immunohistochemical profiles [11-13]. Over expression of p16 gene has been associated with HPV related: cervical cancer, oral squamous cell carcinoma, oropharyngeal squamous cell carcinoma, head and neck cancer (HNSCC) well as non-small-cell lung cancer [11-17]. Also, expression of this global gene has been observed in, invasive breast cancer colorectal cancer and Feline oral squamous cell carcinoma (FOSCC) highly aggressive head and neck cancer in cats [18-20].

Combined results from the HPV-DNA PCR results and p16 immunohistochemistry (IHC), falling into four groups and possibilities of observations can be explained as follows using the knowledge of known mechanism [21]. Obviously, p16 (+)/HPV-DNA (+) confirms the presence of transcriptionally active HPV to elevate p16 gene product as well as undisrupted amplifiable HPV DNA. The p16(-)/HPV-DNA (+) indicates the absence of overexpression of p16 but proves the presence of amplifiable unintegrated HPV DNA. Moreover, p16(+)/HPV-DNA (-) might be an indication of overexpression of p16 but either unavailability of L1 region of viral genome which can be amplified by consensus oligonucleotide primers or other potential mechanism of over expression of p16 which has not been identified so far. Finally, p16(-)/HPV-DNA (-) confirms neither the presence of transcriptional HPV DNA to stimulate the over expression of p16 nor the presence of amplifiable HPV-DNA. Besides, the negativity of both p16 and HPV DNA might suggest the inactivation or deletion of p16 due to a mutation which might initiate the carcinogenesis and the frequency of inactivation was high as 85% in some cell lines, which insinuates the inactivation of this gene can promote uncontrolled proliferation in malignancies [10].

The clinical trials are attempting to establish treatment protocols to place patients in the correct treatment modalities and counselling for better prognosis of HPV related (HNSCC)s [22, 23]. Laboratory diagnosis is needed to differentiate HPV associated cancers from HPV non associated cancers. Various methods are available for HPV detection and HPV DNA detection is being considered as the gold standard of them [24]. However, the detection of high-risk HPV genotypes does not confirm the triggering role of HPV in carcinogenesis. The presence of HPV E6/E7 mRNA only confirms the oncogenic transformation [24-27]. Over expression of p16 is gained much reputation as a prognostic marker in head and neck, lung cancers and colorectal cancers [15, 16, 18]. On the other hand, the p16 IHC positivity confirms the over expression of hosts' p16 tumor suppressor gene, most probably due to the transcriptionally active high-risk HPV [24, 25]. Overexpression of p16 protein is significantly associated with the progression of colorectal cancers by TNM stage and lymph node metastasis and invasiveness of breast cancer, thus p16 IHC seems to be an important biomarker in other cancer types, in addition to well-known cervical and subset of HNSCCs [18, 19].

Inactivation of p16 by methylation is a common event in smoking associated cancers, which differentiate the HPV associated HNSCCs from HPV non associated [28]. P16 over expression can be seen in HPV associated as well as HPV non associated cancers whereas, p16 inactivation can be observed in HPV non associated cancers. Furthermore, p16 immunohistochemistry is recommended to confirm the histological diagnosis of moderate dysplasia and premalignant mimics [29]. In reality, p16 immunohistochemistry can be used as a screening as well as diagnostic test for several cancer types. It has been speculated that p53 responds to high levels of DNA damage whereas, p16 might be able to guards the cumulative effects of minor changes which could be rectified by DNA repair genes [10]. Destruction of malignant tumor clones simultaneously with its oncogenic viral agents could be done by the main treatment modalities: surgery, chemotherapy, and radiotherapy.

Last but not least, the potential utility of p16 over expression has a dual purpose as a diagnostic as well as a prognostic marker in several cancer types. It also indicates the TNM stage of different cancers. In relation with HNSCCs, over expression of p16 is mostly related with a better prognostic outcome with radiotherapy and chemotherapy treatment modalities than p16 negative HNSCCs. The p16 positive IHC tells a story of three related TSGs: over activation of p16 and inactivation of pRb and p53. Inactivation and overexpression of p16 gene is associated with carcinogenesis. In HPV related cancers over activation of p16 can be seen. In contrast, p16 inactivation is associated with HPV nonrelated cancers. In snapshot, p16 established as a diagnostic and prognostic maker in several types of cancers. However, there is a dearth of information on functional potential of p16 ubiquitous gene. There might be unknown mechanisms of overexpression of p16, in addition to the well-known mechanism via high-risk HPV genotypes and known non-HPV-related carcinoma in which basal-like morphology predicts inactivation of Rb protein and diffuse p16 expression in basal-like triple-negative breast carcinoma [30]. This warranted further investigations with large epidemiological studies with cohort study design combined with mechanistic studies using *in vitro* and *in vivo* laboratory experiments.

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