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CIP2A as a Potential Stratification Marker and Target for Tumor Responsiveness to DNA Damaging Therapies

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Abstract

DNA damaging therapies such as irradiation therapy and chemotherapy are used in the treatment of numerous cancer types both definitively and in combination with surgery. However, many cancer types show intrinsic resistance to DNA damaging therapies resulting in failure in tumor eradication and relapse after therapy. Thus it would be very useful to identify novel diagnostic strategies to predict for tumor radio tolerance. Head and neck squamous cell carcinoma (HNSCC) is a common cancer type characterized with great heterogeneity and lack of predictive markers for tumor radio resistance. Recent literature has revealed Cancerous inhibitor of PP2A, CIP2A, as a novel potential diagnostic marker for HNSCC, and other tumors, that show high radio resistance. In particular, we recently identified a functional link between stem cell factor Oct4 and CIP2A in HNSCC cells and demonstrated their potential role in predicting for HNSCC tumor response to radiotherapy. CIP2A´s role in mediating radio resistance *in vivo* has also been recently confirmed by using genetic mouse model. This raises an interesting possibility that diagnostic evaluation of CIP2A in combination with other factors indicative for cancer cell stemness, could be a novel useful diagnostic approach for stratification of HNSCC patients based on their tumor radio resistance. CIP2A could also serve as a target protein for therapeutic radiosensitation.

Keywords: CIP2A; PP2A; Protein phosphatase 2A; Cancer; UT-SCC; Head and neck squamous cell carcinoma; Radiation; Ovarian cancer; CHK1; Therapy response; Oct4; Cancer stem cell; CD24; CD44; MYC

Introduction

DNA damaging therapies such as irradiation therapy and chemotherapy are used in the treatment of numerous cancer types both definitively and in combination with surgery. Cancer cells intrinsic resistance to DNA damaging therapies leads to failure in the successful eradication of cancer [1]. Mechanisms leading to DNA damage tolerance are inadequately known. It has been suggested, that there is a subpopulation of cancer cells, cancer stem cells, that would be responsible for the self-renewal potential of the tumor, and thus eventually, failed therapy response [2].

Head and neck squamous cell carcinoma (HNSCC), the 6th most common cancer in the world, is treated with a combination of radiotherapy, radical surgery and platinum-based chemotherapeutics [3,4]. Recently, radiosensitization using EGFR inhibitors has challenged traditional chemotherapy [5]. While either radical surgery or definitive radiotherapy alone is often a sufficient treatment option for small local tumors, better loco regional control of more advanced disease is achieved with a combination of chemotherapeutics, surgery and irradiation [4]. Currently, there are no available biomarkers for the selection of suitable treatment modalities, which is thus solely based on patient characteristics and TNM staging of the cancer [6]. However, HNSCC has started to emerge as a set of cancers with very different behavioral patterns depending on tumor site, radiotolerance, carcinogen exposure, and stromal reaction [7-9]. These features and their clinical significance are as yet poorly characterized. Copious studies have lately aimed at identification of HNSCC subgroups for both prognostic and therapy stratification purposes [10]. Several potential biomarkers have been identified in HNSCC, but none of the studied biomarkers yet yield sufficient resolution to support their routine clinical use [6]. Promisingly, several clinical trials have recently been launched to study HPV infection as a predictive biomarker for reduced-intensity radiotherapy and EGFR inhibitor response in oropharyngeal HNSCC (ClinicalTrials.gov). The effect of HPV infection on prognosis of other HNSCC subclasses, however, is under debate [11].

Potential Role for CIP2A and Stem Cell Marker Oct4 as Cancer Patient Stratification Marker for DNA Damaging Therapies

Ubiquitous protein phosphatase 2A (PP2A) complexes regulate serine/threonine phosphorylation of diverse cellular signaling pathways related to growth, cell viability, and malignant transformation [12]. An endogenous PP2A inhibitor protein, cancerous inhibitor of PP2A (CIP2A), promotes malignant cell proliferation, and tumor growth in various cancer models [13-16]. Overexpression of CIP2A associates with poor patient prognosis and tumor aggressiveness in virtually all cancer types studied thus far, including breast cancer, prostate cancer, and oral HNSCC [14-15,17,18]. In HNSCC particularly, CIP2A seems to be essential for tumor growth as only around 50% of injections of CIP2A negative HNSCC cells resulted in xenograft tumor growth [13]. In our previous study, we showed, that CIP2A is physiologically involved in the regulation of mouse spermatogonial proliferation and spermatogenesis [19]. CIP2A is expressed in spermatogonial progenitor cells, which are actively proliferating cells capable of self-renewal. Importantly, despite a spermatogenesis defect, CIP2A is dispensable for normal mouse growth and development and the adult mice do not show any apparent pathology [19,20]. Consistent with the important role of PP2A in regulating various phosphorylation-dependent pathways, CIP2A regulates phosphorylation and activity of many critical signaling proteins in cancer, including MYC, E2F1 and mTORC1-dependent growth signaling [15].

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In our recent study [21], we aimed to elucidate the link between CIP2A and other stem cell markers such as octamer-binding transcription factor 4 (Oct4). Oct4 plays a significant role in the stemness regulation of embryonic stem cells and testicular stem cells, and has previously been shown to confer radioresistance and cancer stem cell phenotype in human cancer [8,22]. We demonstrated that, whereas expression of markers for more differentiated cells ceased in mouse testicular cells after *in vivo* irradiation, both CIP2A and Oct4 remained to be expressed at a high level. This suggested that similar to bona-fide stem cell factor Oct4, also CIP2A is expressed in radioresistant population of testicular cells. Further, we showed that Oct4 is an upstream regulator of CIP2A expression in testicular cancer cell lines and embryonic stem cell model, and consistent with the role of CIP2A in regulating MYC, Oct4 depletion led to inhibition of MYC serine 62 phosphorylation [21]. CIP2A and Oct4 were also shown to be coexpressed in testicular cancer patient samples.

Next, we studied similar mechanisms in patient-derived HNSCC cell lines, and showed, that CIP2A and Oct4 expression levels significantly correlated with each other [21]. To study putative HNSCC cancer stem cells, we isolated CD44 and CD24 double positive cells using FACS. Transmembrane adhesion glycoprotein CD44 has previously been linked to stem-like characteristics such as in vivo tumorigenicity of HNSCC cells [23], and CD44/CD24 double positivity was recently reported to identify a chemoresistant cell population capable of self-renewal [24]. Our experiments demonstrated that HNSCC stem-like cells expressed Oct4 and, to a lesser extent, also CIP2A. In conclusion, there is a CIP2A/Oct4 double positive cell population in HNSCC cells, suggesting a potential clinical role for their interaction. We then further studied CIP2A/Oct4 double positivity in a 52 patient tissue microarray. Importantly, tumor CIP2A/Oct4 double positivity significantly predicted for poor patient prognosis after radiotherapy, further suggesting a role for CIP2A and Oct4 interplay in radioresistance of human HNSCC tumors.

As already mentioned, there are currently no sufficiently reliable biomarkers for prediction of therapy response of HNSCC [6]. Small and undisseminated HNSCC tumors are currently treated with either irradiation therapy or radical surgery [4]. Since a significant part of HNSCC tumors prove eventually to be radioresistant [25], there is a desperate need for biomarkers to aid in stratification for therapy. Our results suggest that diagnostic analysis of factors related to stemness of the tumor cells could be useful to characterize tumor radiotolerance. In fact, recently, high expression of stemness marker CD44 in combination with low expression of hypoxia marker HIF-1a, was shown to predict positive outcome in surgically treated non-metastatic stage I oral HNSCC [26].

Our results support the notion that CIP2A play a significant role in the determination of radioresistance of HNSCC cells. Based on our results and role for Oct4 and other stemness factors in mediating radioresistance, it is also feasible to suggest that especially Oct4 driven expression of CIP2A is important for radiotolerance of HNSCC tumors. These conclusions are further supported by recent demonstration that CIP2A is expressed together with its target MYC in crypts of mouse intestinal cells, and that similar to MYC, CIP2A is required for efficient intestinal regeneration in response to both irradiation as well as DNA damaging therapy cisplatin [27]. Importantly, both Oct4-driven CIP2A expression in testicular cancer cells [21], and CIP2A expression in regenerating irradiated mouse intestines [27], promoted expression of oncogenic serine 62 phosphorylated form of MYC. Furthermore, CIP2A expression was shown to define cancer cell response to

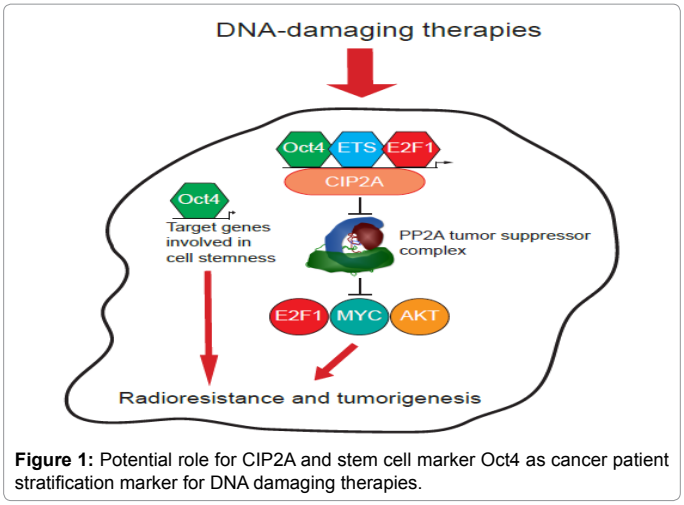

Figure 1: Potential role for CIP2A and stem cell marker Oct4 as cancer patient stratification marker for DNA damaging therapies.

checkpoint kinase Chk1 inhibitors used in clinical cancer trials [16]. Moreover, high CIP2A expression predicts for poor patient survival of ovarian cancer patients after treatment with DNA damaging platinum-based agents [28], and to promote breast cancer cell resistance to doxorubicin [29]. Thereby, it is very plausible that, in addition to our recent indications of importance of CIP2A in radiotherapy resistance, CIP2A may play a more general role in response to DNA damaging therapies. We are currently validating the role of CIP2A, Oct4 and other potential HNSCC stemness markers in their potential to predict for tumor radioresponse in a larger patient cohort with an ultimate goal to characterize potential marker combinations to be used for clinical patient stratification. Moreover, we aim to test whether inhibition of CIP2A function could be a potential novel approach to increase tumor response to localized radiation therapy in HNSCC and other cancers (Figure 1).

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