Genetic profiling and *in vitro* characterization of *OTX1* functional role on bladder cancer stem cells

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Background of the research

Bladder cancer has the highest cost per patient among all cancers due to the high risk of recurrence and the need for life-long routine monitoring as well as therapy. Cancer stem cells (CSCs) are hypothesised to be linked to long-term recurrence risk due to its ability to repopulate the entire tumour population in a small number of cells. In addition, CSC as according to the stem cell model, has the property of self-renewal. Thus, only CSC has sufficient time to accumulate adequate genetic mutations that drive the acquisition of resistance against chemo- and radiation-therapy leading to initiation of recurrence. Recently, *OTX1* has been shown to be involved in the differentiation of breast CSCs into differentiated cancer cells. The gene is also reported to be involved in other cancers such as subset of B cells in aggressive non-Hodgkin lymphomas, lung cancer and most recently in bladder cancer. Although the involvement of OTX1 in cancers is demonstrated by limited number of studies, the gene remains to be an interesting candidate for further investigation. We propose that *OTX1* may be responsible for the differentiation of CSC and also promote tumour aggressiveness. We plan to establish the expression and localisation patterns of *OTX1* gene for Nigerian bladder cancer patients and correlate with clinical characteristics such as stage and grade; risk of recurrence; disease

progression and age. To understand the role of *OTX1* in bladder cancer stem cells, the gene will be overexpressed/knockdown in non *OTX1* expressing and *OTX1* expressing CSCs, respectively, and the resulting phenotypic effects will be compared.

Statement of the problem

Recently, overexpression of *OTX1* gene has been described in breast cancer, lymphomas and medulloblastomas. However, the gene is also reported to be hypermethylated (gene silencing) in breast cancer as well as lung and bladder cancers. Due to limited literature reporting the functions of the gene in cancers, the reason(s) for *OTX1* to be overly expressed or silenced in certain types of cancers have yet to be ascertained.

Objectives of the research

1. To establish *OTX1* protein expression and localisation patterns for Nigerian bladder cancer patients.
2. To assess the correlation between *OTX1* protein expression clinical characteristics (stage and grade; risk of recurrence; disease progression and age).
3. To determine the role of *OTX1* gene in bladder cancer stem cells phenotypes.

Research questions

a. What is the spectrum of *OTX1* protein expression in Nigerian bladder cancer patients?
b. Is the protein expression of *OTX1* correlates to clinical characteristics of bladder cancer?
c. What are the phenotypic functions of *OTX1* gene in bladder cancer stem cells?

Literature review

Bladder cancer also knowns as urothelial cell carcinoma (UCC) is the ninth most common cancer in the world (Antoni *et al.*, 2017), accounting for 4.7% of all the new cancer cases that translated to an incidence of 79,030 new cases/year with an estimated death of 16,870 in 2017 (Institute, 2017). In Nigeria, bladder cancer is the 2nd most common cancer of the urogenital system after prostate cancer in Nigeria (Takure *et al.*, 2015). The long-term risk of recurrence is linked to the existence of Cancer Stem Cells (CSCs). In accordance with the CSC hypothesis, CSCs possess self-renewal capability that is able to regenerate tumours with phenotypic heterogeneity found in initial tumours. Recently, it has been reported that the human *OTX1* gene is overexpressed in ductal and lobular invasive breast cancer (Terrinoni *et al.*, 2011). Interestingly, *OTX1* was very recently reported by a study to be hypermethylated in bladder cancer patients (Beukers *et al.*, 2013). However, the study only reported the methylation status of the gene and did not perform further experiments to elucidate the role of *OTX1* in bladder cancer. To date, there are only 6 studies that investigate the involvement of *OTX1* in cancers and none of them describe the phenotypic function of the gene in tumour progression and recurrence. We believed that investigating the role of *OTX1* in bladder cancer will improve our understanding of the relationship between CSCs and the aggressiveness of tumour as well as recurrence.

Theoretical framework

Regulatory processes in cancer are understood through a system biology framework. Hence, to evaluate the role of *OTX1* in bladder cancer stem cells, the gene will be overexpressed or knockdown in non *OTX1* expressing and *OTX1* expressing CSCs, respectively, and the

resulting phenotypic effects will be compared in other to understand the molecular mechanism underlying the pathogenesis of the disease contributed by *OTX1* gene. By this way, bladder cancer misregulation, recurrence and resistance to therapy could be solved.

Research methodology

FFPE tissue and data collections: Retrospective collection of archived formalin-fixed paraffin-embedded (FFPE) tissue blocks will be collected from the Abubakar Tafawa Balewa University Teaching (ATBU), Hospital, Bauchi.

Cell culture of bladder cancer cell lines: Six bladder cancer cell lines of varying degree in aggressiveness and also resistant to chemotherapy or radiotherapy will be maintained in supplemented RPMI-1640 media at 37°C in a humidified incubator at 5% CO_2.

Immunohistochemistry (IHC) analysis: The expression pattern of *OTX1* proteins will be evaluated by IHC assay.

Isolation of cancer stem cells by FACS: Cancer stem cells population will be isolated independently from 6 bladder cancer cell lines of varying degree in aggressiveness and also resistant to chemotherapy or radiotherapy

Gene expression analysis (RT-qPCR): Total RNA extraction for various bladder cancer cell lines and FFPE tissues will be performed using the RNeasy Mini kit and RNeasy FFPE kit (Qiagen, Germany). The RT-qPCR assays will be performed in triplicates for each sample on the Rotor-Gene 6000 by QuantiNova SYBR Green PCR (Qiagen, Hilden, Germany).

In vitro modulation: The functional effects of *OTX1* gene on various isolated bladder cancer stem cells phenotypes will be investigated *in vitro.*

Phenotypic assays: Assays such as cell cycle analysis, cell proliferation, cell adhesion assay, apoptosis and migration will be carried out on the bladder cancer cells.

Data analysis: Correlation between *OTX1* protein expression patterns and clinical characteristics will be tested by chi-square test with *P*-values of 0.05 or less to be considered statistically significant. Survival analysis will be performed by using Cox regression analysis in R package environment.

Expected results

1. ***Research Publications***

   The outcome from this study will be published in a high-impact factor peer-reviewed scientific journal.

2. ***Specific or Potential Applications***

   By understanding the phenotypic functions of *OTX1* gene in bladder cancer stem cells, potential translational applications can be designed and validated. *OTX1* may potentially be used in future diagnostic applications to serve as a biomarker for CSC in aggressive bladder cancer.

3. ***Number of PhD and Masters (by research) Students***

   This project is expected to train 1 Master student.

Innovation

Findings from this study will be the first to report protein expression and localisation patterns of *OTX1* gene for Nigerian bladder cancer. Moreover, the role of *OTX1* gene in bladder cancer stem cell phenotypes will be revealed.

Reference

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