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Silencing of FTS increases radiosensitivity by blocking radiation-induced Notch1 activation and spheroid formation in cervical cancer cells

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ABSTRACT

Increasing evidence(s) suggests that cancer stem cells (CSC) in tumours contribute to radio-resistance and recurrence. Notch plays an important role in the maintenance of CSC in many cancers including cervical cancer. Previously, we have reported the role of Fused Toes Homolog (FTS) in conferring radioresistance in cervical cancer cells *in vitro* and human subjects. The present study investigated the regulatory role of FTS in Notch signaling and maintenance of CSC upon irradiation of cervical cancer cells. The expression of Notch1, 2, 3, cleaved Notch1 and its downstream target Hes1, and spheroid formation was increased by irradiation. Silencing of FTS prevented the radiation-induced increase in the expression of Notch signaling molecules and spheroid formation. Immunoprecipitation showed FTS binds Notch1 and Hes1. Also *in silico* structural analysis identified putative residues responsible for the binding between FTS and Notch1. Spheroid formation and the expression of CSC markers, Nanog, Oct4A and Sox2 were greatly reduced by combining silencing of FTS and radiation. Taken together, these results suggest that FTS is involved in the regulation of irradiation-induced Notch signaling and CSC activation and can be used as a target to increase radiosensitivity in cervical cancer.

1. Introduction

Cervical cancer is the third most common cancer in women worldwide [1]. Human papilloma viruses (HPV), such as HPV-16 and 18 play a vital role in development and progression of cervical cancer [2]. RT is a standard treatment for locally advanced cervical cancer with or without chemotherapy. In spite of the initial response, about one fourth of the patients suffer from recurrence of cancer after the treatment. Therefore, radioresistance is one of the important problems to be solved to improve the survival and life quality of cervical cancer patients. Although several mechanism(s) of radioresistance have been explored during the past several decades, recent accumulating evidences support that CSCs and their signaling pathways play important roles in regulating radiation response and radioresistance [3]. Ionizing radiation has been shown to activate the Notch signaling pathway, resulting in increased number of CSCs and increased radioresistance [4].

Notch signaling is involved in cellular proliferation, differentiation, cell-to-cell communication, cell survival, adult stem cell self-renewal, apoptosis and tumourigenesis [5]. More importantly, Notch pathway plays a main role in the maintenance of cancer stemness and its consistent activation leads to disease progression and metastasis [6]. Notch is expressed abundantly in many tumours including cervical cancer and has been an attractive therapeutic target for the treatment of cancer [7]. There are four members of Notch receptor (Notch1–4). Even though Notch1, 2, 3 and 4 are expressed in mammals, Notch1 and 3 are abundantly expressed in cervical cancer and mediate the progression of cervical cancer [8,9]. The receptors are activated by one of the known five Notch ligands [Jagged1 (JAG1), Jagged2 (JAG2) and Delta-like 1, 3, 4 (DLL1, DLL3, DLL4)] [10]. Proteolytic cleavage of Notch intracellular domain (NICD) by γ-secretase initiates Notch-dependent nuclear signaling. NICD translocates to nucleus and activates the Notch target genes such as Hes1. Targeting Notch signaling by using γ-secretase inhibitors (GSI) have been suggested as an effective method to treat CSCs and tumour endothelial cells [11,12].

Sphere derived CSCs express high amount of stem cell markers [13]. Radiation-induced Notch activation and maintenance of stemness confers radioresistance in hepatocellular carcinoma [14], oral squamous cell carcinoma [15] and glioma cells [16]. Targeting Notch signaling by specific GSIs improved the efficacy of RT [16]. Cisplatin, a standard chemotherapeutic drug, increases Notch1 cleavage and then activates stem cell markers, tumour regeneration and metastasis in lung cancer [17] and ovarian cancer [18].

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