Chapter 1

Introduction
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Oral cancer is the sixth most common cancer worldwide [1]. In India, extensive tobacco usage in various forms make it the leading type of cancer among males and third most common cancer in females [2,3]. In the last decade, developments in the field of oral cancer treatment have resulted only in a modest improvement in survival rate [4]. The treatment modalities of oral cancer are based on various factors including disease stage, access to the tumor site, age and physical status of patient. Although, surgery is choice of treatment in early stages; radiotherapy also holds an integral place either alone or as an adjuvant mode of treatment with chemotherapy [5,6].

Standard radiotherapy protocol for oral cancer involve daily exposure of 2Gy fractionated radiation dose for few weeks and in this way patients receive a cumulative dose of 50Gy to 70Gy during the course of fractionated radiotherapy [7,8]. Fractionated radiation kills fast dividing tumour cell population with decreased effects on surrounding normal tissues. This method provides time for normal cells to repopulate and recover while diminishing tumour cells that have aberrantly activated signalling pathways [9,10]. Such radiation therapy is the standard adjuvant treatment for oral cancer, but it often fails as the cancer cells become refractory to radiation and develops radioresistance [11]. The development of radioresistance is a major hurdle in the efficacy of radiotherapy in oral cancer patients and in order to make radiotherapy more effective, it is important to explore the phenotype of radioresistant oral cancer cells.

Previous studies from our lab have demonstrated, the altered expression of Bcl-2 family members and the over expression of anti-apoptotic splice variant of Mcl-1 in human oral cancers [12]. Further, the role of anti-apoptotic Mcl-1 in cellular radio & chemoresistance was also demonstrated [13,14]. Such anti-apoptotic molecules can be one of the factors
responsible for radioresistance, but development of radioresistance is a complex phenomenon, involving several proteins from different cellular processes. Therefore, global molecular profiling of radioresistant oral cancer cells would be of help in identifying radiation resistance related molecules and thus contribute to a better understanding of oral cancer treatment response.

Also, it has been observed that failure of cancer therapy has been associated with Epithelial to Mesenchymal transition (EMT) reactivation and enrichment of cancer stem cell like (CSCs) population within the tumors that are undergoing fractionated radiotherapy [15,16]. EMT is a complex order of events by which epithelial cells acquire mesenchymal traits by losing their polarity, cell-cell contact and reorganize their cytoskeleton [17]. The cells undergoing EMT acquire migratory properties to invade and metastasize due to loss of epithelial markers like E-cadherin, Desmoplakin and gain of mesenchymal markers such as Vimentin and N-cadherin [18,19]. Several reports suggest the role of these EMT markers in context to oral cancer [20,21] and expression of such EMT characteristics in chemoresistant model systems has been reported [22,23]; but its role in radioresistance and especially in context to oral cancers are largely unknown [24,25].

Similarly, studies have revealed that small heterogeneous population of cancer stem like cells within the tumor possess the property of self-renewal and differentiation [26]. Accelerated repopulation of tumor cells during or after radiotherapy treatments are among the well documented causes of treatment failure. Studies indicate that ionizing radiation can enhance the small population of cells expressing stem cell like markers [27]. These surviving stem cells like population may be responsible for resistance to cancer therapy [28] and are the focus of recent treatment modalities in oral cancer [29].
Further, the biomedical applications of Raman spectroscopy has already been investigated in pathological conditions and cancer detection [30]. The Raman related study with regard to radiation induced biochemical changes will be helpful in identifying distinct spectral signature profiles of the established radioresistant cells.

Therefore, in the present study we explored the differential profile and characterized the properties of radioresistant oral cancer cells. We first established oral radioresistant sublines from their parental cell lines of different oral subsites by treating with clinically admissible low dose fractionated ionizing radiation. These radioresistant sublines were generated by completing the course of radiotherapy that is generally given to the oral cancer patients during the course of their treatment. The proteomic and transcriptomic studies were carried out on the resulting radioresistant sublines in order to investigate their differential molecular profile. In addition, acquired radioresistance related properties like - EMT, cancer stem cells and Raman spectroscopic studies on these parental and established radioresistant cells were also carried out. The outcome of the study will help in understanding radiotherapy response leading to better treatment possibilities for oral cancer patients.