RESEARCH ARTICLE

Adenocarcinoma of the Prostate: Molecular Strategies for Improving the Therapeutic outcome for Radiation Therapy

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Abstract

With process improvements in treatment including image guidance and intensity modulation, radiation therapy remains an important component of care for patients affected with prostate carcinoma. Although radiation therapy coupled with hormone therapy can provide exceptional cure rates in patients with low and intermediate risk factors, more progress is needed for patients with high risk of failure. These patients often can present with large volume disease in the prostate with unfavorable Gleason grade and risk for disease at and beyond the prostate capsule. These patients are predominantly treated with radiation therapy for local treatment. Developing strategies to augment control with radiation therapy in parallel to hormone therapy is important for the next generation of prostate cancer patients. This paper reviews potential molecular pathways that can sensitize prostate cancer cells to radiation therapy and potentially improve the therapeutic index for patients with this disease.

Introduction

Prostate carcinoma remains a significant issue for oncology clinical care. The disease affects a large percentage of our population and can have a disproportionate malevolent outcome on vulnerable populations[1]. While favorable outcomes are seen in patients with low-risk disease with local therapy including surgery and radiation therapy in multiple formats, a significant percentage of men present with unfavorable and high-risk features including high Gleason grade, neuroendocrine features, and nodal involvement. With clinical nihilism now seen in PSA screening and lassitude in surveillance follow up, more high-risk patients are again seen at younger ages. It is important to pursue process improvements in therapy.

Radiation therapy remains an important component to the care of patients with this disease. Process improvements in radiation therapy including image guidance and intensity modulation have provided a significant increase in tumor control and limitation in normal tissue injury. Cure rates with radiation therapy have been improved with the addition of hormone therapy, therefore providing a strategy to offer better patient care with improved long-term outcome. Hormone therapy may function through multiple mechanisms. While it is common to associate the success of hormone therapy with testosterone interruption, hormone may also interfere with tumor cell adhesion serving to inhibit tumor growth and metastasis[2]. Therefore, identifying multiple pathways including hypoxia conditions, DNA damaging as well as signaling pathways become the important vehicles for interaction to augment the success of radiation therapy.

In this manuscript, we review recent advances in the understanding of the molecular biology of prostate cancer and identify strategies for optimizing patient care moving forward.

Role of Adhesion Molecules

Although we traditionally associate the mechanism of hormone therapy with interruption of the testosterone pathway, hormone therapy contributes to prostate cancer cell death through multiple mechanisms, many are not yet well characterized. In order to proliferate, cancer cells must adhere to a structure and in turn develop a blood supply for support[3,4]. Often cells adhere to stroma for structural support and the development of tumor associated angiogenesis. Although promising therapies have been directed to angiogenesis inhibition, often these therapies serve to stabilize established tumor blood vessels and not always generate a predictable response to therapy. It may prove to be more important to inhibit the initial development of tumor vascularity at the initiation of angiogenesis to abate tumor cell growth and development.

We have demonstrated in early publications that bicalutamide inhibits androgen mediated adhesion of prostate cancer cells when exposed to radiation therapy [5]. In a series of experiments our group was able to study the role of bicalutamide on cell adhesion properties in LNCaP prostate cancer cells. LNCaP cells were stimulated with androgen prior to radiation treatment with 0, 5, 10, and 15 Gy. Cell adhesion to fibronectin was measured to ascertain the role of androgen in integrin mediated prostate cancer cell adhesion with flow cytometry used to define the expression of integrin subtypes in LNCaP cells exposed to both androgen and radiation

therapy. LNCaP cell adhesion to fibronectin was significantly increased by stimulation by androgen alone and with androgen coupled with radiation therapy. The stimulation of cell adhesion was abrogated with the application of bicalutamide. In parallel, cells exposed to radiation at higher dose likewise demonstrated an increase in $\alpha_v\beta_1$ integrin expression which was likewise abolished with the addition of bicalutamide. The data supported the premise that bicalutamide played an important role in abrogating increased cell adhesion seen in LNCaP cells with androgen and surprisingly with radiation therapy. While reasonable to assume that the increase in cell adhesion seen after radiation therapy can be an immediate response to injury, it is worrisome to think on balance that the initiation of therapy can have an initial deleterious impact on outcome by supporting tumor cell adhesion and support growth and development of disease with the initiation of management. Therefore, the use of hormone therapy as radiation therapy is initiated may be an important aspect to clinical care transparent to the duration of hormone therapy which requires further study. Likewise, the mechanism of action of bicalutamide is not clear, nevertheless one of the roles supported by hormone therapy is the impact of hormone on tumor cell adhesion. It will be important to explore the mechanism of action in greater depth to optimize both timing, dose, and duration of hormonal therapy and its role as a co-partner to radiation therapy [2,6,7]. Identifying cell adhesion specific therapy in lieu of hormone treatment may also serve to reserve hormone therapy for first or secondary treatment failure[4,8-12]. This would potentially limit risk of the cardiovascular, muscular-skeletal, and neurocognitive sequelae of hormone therapy.

MAP kinase pathway and the role of radiotherapy

Extracellular signal regulated kinases (ERK) are one of major classes of mitogen-activated protein (MAP) kinases that transduces and activates a variety of extracellular signals to regulate cell functions involving proliferation, differentiation and cell death [13,14]. ERKs are well known as key signaling transducers that mediates cell proliferation in response to the growth factor, much evidence shows that ERK pathway, like JNK and p38, can be activated by environmental stresses such as reactive oxygen species [15] and radiation[16,17]. In prostate cancer patients, an elevated level of phosphorylated ERK1/2 has been found in castration resistant prostate cancer as compared to untreated primary prostate cancer[16,17]. Signal transduction-based therapy can, by disrupting the MEK/ERK/c-Myc axis, reduce human prostate cancer (PCa) radioresistance caused by increased c-Myc expression in vivo and in vitro and restore apoptosis signals [18]. This suggests pharmacologic targeting of the MEK/ERK pathway may be a viable treatment strategy adjunct with radiotherapy for patients with refractory metastatic prostate cancer.

Our laboratory has been fortunate to study the impact of ERK1 and ERK2 expression and the impact of molecular expression products. ERK1 and ERK2 have multiple functions in tumor cells including influencing cell growth/development and senescence. The pathways also have influence in the function of mitochondria. We have been able to grow and develop cells from prostate cancer cell lines (DU145 and LNCaP) lineage that are resistant to radiation therapy [19]. In a series of experiments, we were able to isolate the maintain cells that survived long term and

regrowth after 40 Gy of radiation therapy. We have referred to them as DUIR or LI. While both cells have the capability of expressing neuroendocrine biomarkers, DUIR has demonstrated epithelial-mesenchymal transformation (EMT) in addition. Both cell lines have features associated with modern defined malevolent subtypes of prostate cancer. These cells express significant enhancement of ERK1 and ERK2 phosphorylation and are resistant to radiation therapy. Treating DUIR cells with ERK inhibition (sh-RNA) abolished radiation resistance and returned DUIR cells back to the sensitivity profile of their cell of origin [20].

This is an important finding as it begins to place emphasis on patient care management defined by molecular expression products for radiation therapy. There are defined cohorts of patients at high risk that do not have the same clinical outcome to their low and intermediate risk counterparts, and this is the group of patients we need to target for process improvements in care. Further characterization of molecular profiles will increase our knowledge in this important area and serve to further improve patient care by potentially tailoring therapy to specific targets personalized to each patient.

Hypoxia-inducible factor 1α (HIF-1α) and radiotherapy resistance

Hypoxia-inducible factor (HIF) is a transcriptional complex that plays a central role in mammalian oxygen homeostasis[21]. Among two subunits of HIF, HIF-1α and HIF-1β [22], HIF-1α is a critical regulator in cells under multiple stressful conditions [23]. Almost half of the all solid tumors contain hypoxia subregions with various sizes and extents [24]. Meanwhile, in advanced and castrate resistant disease, HIF-1α is a biomarker for therapeutic resistance. PCa is one of the major malignancies within which hypoxia has a significant impact on treatment resistance and metastasis [25,26]. HIF-1α is associated with gene expression in patients and is found to be correlate with higher Gleason score, T stage, and malevolent outcome in PCa [27,28]. The findings that HIF-1α expression with greater frequency in patients with more advanced disease suggests the possible need for therapy beyond the application of traditional hormone/radiation therapy [29]. There is increasing information that expression of HIF-1α is associated with resistance to chemotherapy and due to activation in areas of hypoxia, may contribute to resistance in radiation therapy [30]. The mechanism of therapeutic resistance is likely diversified in disease origin and includes adaptive changes within cells to hypoxia including glycolysis, limitation of vascularity including drug concentration in areas of disease, and expression of other molecular pathways associated with resistance including angiogenesis epithelial-mesenchymal transition [31-33] and possibly associated with regulation of mammalian target of rapamycin (mTOR) signaling pathway [34]. A retrospective analysis of two randomized radiotherapy trials and one surgical cohort study indicates increased HIF-1α expression is a significant predictor of biochemical failure after radiotherapy or surgery for prostate cancer [35].

The relationship of HIF and therapeutic resistance for radiation treatment in prostate cancer is not well understood. This is an important issue to study. Although there has been considerable progress in the application of radiation therapy to prostate cancer patients, identifying higher risk patients and studying how to improve outcome in

conjunction with radiation therapy become important. In a manner similar to the use of hormone therapy, adding strategic therapy with radiation therapy has the potential of improving outcome for patients thought to be at higher risk for failure. Our laboratory has investigated this question. Preliminary data in prostate cancer cells has shown that cells resistant to radiation therapy expressed HIF-1α indicating this as an important target for future investigation.

PARP inhibition and radiosensitivity

Poly (ADP-ribose) polymerase-1 (PARP-1) is a ubiquitous enzyme found mostly in the nucleus. PARP1 plays an important role in the base excision repair (BER) pathway for DNA single strand breaks (SSBs). It catalyzes the covalent binding of polymers of ADP-ribose (PAR) moieties on itself and its target nuclear proteins from donor nicotinamide adenine dinucleotide (NAD^+) molecules [36]. Modern sequencing techniques have identified DNA repair deficits in patients with advanced prostate carcinoma and those with the presence of BRCA mutations. In metastatic castration resistance prostate cancer (CRPC), genomic aberrations have been shown to interfere with DNA repair [37,38]. PARP-1 inhibitors are now an important component to the treatment of advanced and castrate resistant prostate cancer [39-41]. Coupled with next generation androgen therapy, inhibition of DNA repair adducts appear to be an important component to patient care for patients with advanced disease and those that require therapy independent of androgen pathways. Serval clinical trials indicate that PARP inhibitor can enhanced survival in PCa patients. In a recent Olaparib trial, for example, among men with metastatic CRPC who had tumors with at least one alteration in *BRCA1*, *BRCA2*, or *ATM* [42,43] and whose disease had progressed during previous treatment with a next-generation hormonal agent, those who were initially assigned to receive Olaparib had a significantly longer duration of overall survival than those who were assigned to receive enzalutamide or abiraterone plus prednisone as the control therapy [44].

Radiation therapy damages DNA and cellular response to radiation has been shown to correlate with the proportion of cells in replication [45]. Therefore, agents that serve to further inhibit DNA repair may be an important addition to radiation treatment in order to determine if selected addition of targeted therapy with radiation therapy can improve outcome for patients. It is well known that tumors contain a higher proportion of replicating cells than normal tissues, and the cell-cycle checkpoint responses of these cells are often defective [46]. Radiation-induced DSBs are often repaired through nonhomologous end joining (NHEJ). Ionizing radiation in the clinical treatment of cancer generates mainly SSBs rather than DSBs. The major effect of PARP inhibition is to delay the repair of SSBs. Since DSBs are the most important cytotoxic lesions, the effect on SSB repair may have a minimal impact on the survival of nonreplicating cells. In contrast, PARP inhibition increases radiosensitivity of proliferating cells. Thus, PARP inhibitors may improve the outcome of radiotherapy in tumors by promoting damage in highly replicating tumor cells while having limited impact on noncycling normal tissues that have dose-limiting late damage in response to radiotherapy. Our laboratory has begun to investigate this point to see if the application of PARP inhibition to radiation therapy can serve to increase tumor cell kill (Fig 1). There is preliminary

evidence that PARP inhibition increase cell kill with radiation therapy is previously resistant cell lines.

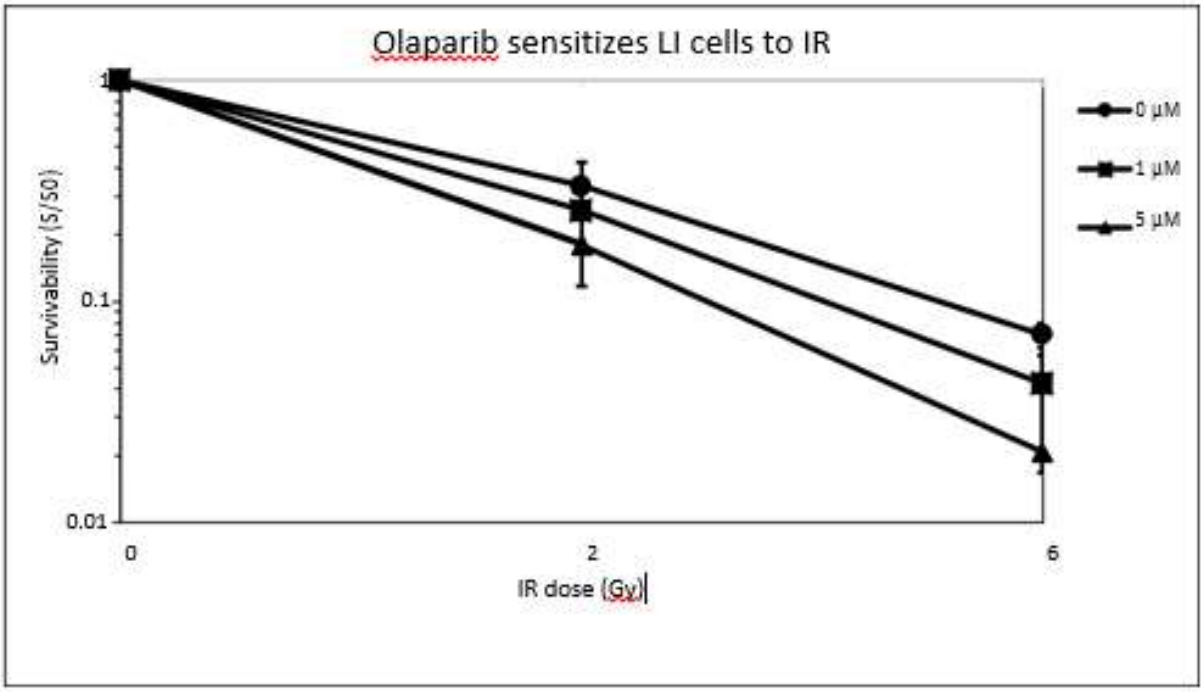

Fig. 1. The clonogenic assay was performed when cells were exposed up to 6 Gy ionizing radiation. Cells were cultured in the presence of Olaparib (Selleckchem **Co.,** Houston, TX) for 48 hours before exposed to ionizing radiation (IR). Surviving fraction is expressed by a log scale. Colony formation was scored two weeks post radiation.

Conclusion

Radiation therapy remains an important pathway to care for patients with prostate cancer and plays an increasingly important role in patients with high risk of treatment failure. Identifying agents that can increase cell kill in conjunction with radiation therapy is an important next step for progress in therapy including situations that will apply advanced technology radiation therapy techniques to the treatment of patients with oligometastatic disease. Our laboratory has evaluated opportunities to increase tumor cell kill in prostate cancer with radiation therapy. Therapies directed to cell adhesion, ERK and mTOR, HIF, PARP pathways and apoptosis associated molecules appear to improve cell kill with radiation therapy and may prove to be good strategies to apply in clinical trials moving forward in selected patients with these biomarkers as expression products.

The cost of additional therapy will decrease as they become more popular and used at an enterprise function. The therapies will reduce failure and accordingly decrease cost as secondary relapse therapy becomes less necessary.

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