

Prostate Cancer

ERK ACTIVITY FACILITATES ACTIVATION OF THE S-PHASE DNA DAMAGE CHECKPOINT BY MODULATING ATR FUNCTION

Wu D, Chen B, Parihar K, He L, Fan C, Zhang J, Liu L, Gillis A, Bruce A, Kapoor A, Tang D

ABSTRACT: Although Erk kinase has been recently reported to function in the DNA damage response, the mechanism governing this process is unknown. We report here that hydroxyurea (HU) activates Erk via MEK1, a process that is sensitized by a constitutively active MEK1 (MEK1Q56P) and attenuated by a dominant-negative MEK1 (MEK1K97M). While ectopic MEK1Q56P sensitized HU-induced S-phase arrest, inhibition of Erk activation via U0126, PD98059, and MEK1K97M attenuated the arrest, and thereby enhanced cells to HU-induced toxicity. Taken together, we demonstrate an important contribution of Erk to the activation of the S-phase DNA damage checkpoint. This can be attributed to Erk's regulatory role in modulating ATR function. Inhibition of Erk activation with U0126/PD98059 and MEK1K97M substantially reduced HU-induced ATR nuclear foci, leading to a dramatic reduction of γ H2AX and its nuclear foci. Reduction of MEK1 function by a small interference RNA (siRNA) MEK1 and ectopic MEK1K97M significantly decreased HU-induced γ H2AX. Conversely, ectopic MEK1Q56P enhanced γ H2AX foci. Furthermore, immunofluorescent and cell fractioning experiments revealed cytosolic and nuclear localization of ATR. HU treatment caused the redistribution of ATR from the cytosol to the nucleus, a process that is inhibited by U0126. Collectively, we show that Erk kinase modulates HU-initiated DNA damage response by regulating ATR function.

THE ANATOMIC AND PATHOLOGIC CHARACTERISTICS OF IRRADIATED PROSTATE CANCERS MAY INFLUENCE THE ONCOLOGIC EFFICACY OF SALVAGE THERAPIES

Huang WC, Shayegan B, Kuroiwa K, Fernando J, Bianco F, Scardino PT, Eastham JA

PURPOSE: Recurrent or radioresistant prostate cancer occurs in approximately 30% of men receiving primary radiotherapy. For men who are candidates for local salvage therapy, the oncological efficacy of ablative therapies may be affected by the anatomical and pathological features of cancers within irradiated prostate glands. We characterized and mapped the prostate cancers in our series of whole mount salvage radical prostatectomy specimens.

MATERIALS AND METHODS: A total of 47 salvage radical prostatectomies were performed at our institution between 2000 and 2004. Detailed pathological data, including the anatomical distribution of cancers, were obtained from 46 whole mount salvage radical prostatectomy specimens.

RESULTS: A total of 70 cancer foci were identified in 46 specimens. Of the specimens 93% had cancer foci at the apex. The median minimum cancer-to-urethra distance was smallest at the apex (4.1 mm) and greatest at the base (13.8 mm). More than 65% of patients had cancer 5 mm or less from the urethra and 7% of patients had cancer directly involving the urethra. Nearly half of all patients had evidence of extraprostatic disease.

CONCLUSIONS: The anatomical and pathological features in our study demonstrate that a significant portion of irradiated cancers are pathologically advanced and distributed in regions of the prostate (apical and periurethral) which are at risk for undertreatment using current ablative therapies. Our findings raise serious concerns regarding the oncological efficacy of such treatment modalities. Long-term studies without the use of hormonal therapy are needed to determine the oncological efficacy of salvage ablative therapies in patients with radiorecurrent or resistant prostate cancer.

PREDICTION OF THE RESPONSE OF PROSTATE CANCER PATIENTS TO RADIATION USING SURROGATE TISSUES

Pinthus JH et al.

INTRODUCTION: Radiation therapy is standard treatment for prostate cancer (PC). Postulated mechanism of action for radiation therapy is via the generation of reactive oxygen species (ROS). Using the current regimen of high dose conformal radiation, treatment failure occurs in 45% of patients with locally confined disease. It is likely that, in addition to individual patient risk factors, intrinsic differences in cellular radio-sensitivity exist which explains the diversity in response between different patients. In previous work we demonstrated the effects of the individuals' androgenic milieu on the basal levels of ROS (bROS) in the PC that leads to relative resistance to irradiation through the over-expression and increased activity of several stress kinases and that of anti-oxidative enzymes (adaptation molecules). Here we demonstrate for the first time that the same effects can be observed in salivary glands and hair follicles of the same individual with PC.

METHODS: The salivary glands and hair follicles were selected since both tissues are androgen regulated and since their histological structure and hemostasis are analogous to the prostate. Eighteen adult Sprague Dawley male rats weighing 200-250g were randomized into 3 groups of 6 rats each: Group A (assigned A- to indicate lack of endogenous and exogenous androgens): underwent bilateral trans scrotal orchiectomy. Group B (assigned A+ to indicate presence of both endogenous and exogenous androgens): underwent sub-cutaneous implantation of slow release testosterone pellet. Group C (assigned as C had their endogenous production of testosterone i.e. testes left intact) underwent sham procedure. These 3 groups thus represent 3 different levels of host androgens-complete absence (A-), normal range (C) to increase levels (A+). The rats were sacrificed after 48 hours. The tissues (skin, prostate, salivary gland) were removed immediately and cut into two halves for histological examination and for tissue lysate preparation. The fixed tissues were embedded in paraffin and were then reconstructed into a pre-planned tissue microarray (TMA) platform. Tissues were examined for the expression of Bros and oxidative stress adaptation markers using specific immunohistochemistry, immunoblotting and enzymatic assays.

RESULTS: The basal redox status in the prostate, salivary glands and hair follicles in the same hosts were affected in a similar manner by the androgenic milieu. Consequently, In each individual host all 3 tissues had the same expression pattern of adaptation markers to radiation such as clusterin, p-p38, catalase and others which was significantly higher in groups B&C as compared to group A. The expression of these markers was shown to influence the response to radiation.

CONCLUSIONS: The individuals' sensitivity to oxidative stress based therapies is dictated by the individuals' redox status and expression of adaptation molecules. This is an androgen-regulated phenomenon. In each individual with hormone sensitive tumor, the salivary glands and the hair follicles serve as a surrogate tissues to pre-determined ones own potential sensitivity to radiation in a minimally or non-invasive method.

NADPH OXIDASES AS A POTENTIAL TARGET FOR PROSTATE CANCER RADIO SENSITIZATION

Pinthus JH, Lu JP, Bryskine I, Monardo L, Singh G

INTRODUCTION: The generation of toxic oxidative stress is the core mechanism for radiation therapy (RT). Despite contemporary usage of high dose RT for localized prostate cancer (PC) long term failures are still substantial but can be reduced by adjuvant androgen deprivation therapy (ADT). We have previously shown that ADT radio sensitizes PC cells through the modulation of oxidative stress (Neoplasia. 2007 Jan;9(1):68-80). In attempt to spare potential side effects of adjuvant ADT we examined if the inhibition of reactive oxygen species (ROS) generation by NADPH oxidases (NOX), a membrane multimere superoxide generating protein complex, could potentially replace the need for ADT.

METHODS: We compared the effects of a synthetic androgen (R1881), an androgen receptor (AR) blocker (bicalutamide) and 2 different NOX inhibitors: apocyanine and DPI on the production of ROS in the androgen responsive 22rv1 human PC cells. The expression of 2 core NOX subunits- p22phox and gp91-phox, ROS production and the radio-sensitivity of the cells (colony formation assay following single exposure to 3Gy) were compared between these treatments.

RESULTS: 22rv1 cells that were treated with physiological concentration of androgens (10⁻⁸M R1881) had increased specific production of ROS by NOX and had reduced sensitivity to radiation compared to 22rv1 cells that were deprived from androgens. The addition of bicalutamide, apocyanine or DPI to R1881 reduced ROS production and restored the sensitivity of the cells to radiation. Androgens increased the expression of the p22-phox and gp91-phox subunits of NOX as compared to treatment with androgens + bicalutamide or by androgen deprivation.

CONCLUSIONS: Androgens regulate the expression of the p22-phox and gp91-phox NOX subunits in 22rv1PC cells. Increased generation of ROS by NOX induces radio resistance in 22rv1 PC cells but this can be ameliorated by the use of ADT or NOX inhibitors.

INTER-RELATED EFFECTS OF ANDROGENS, FATTY ACIDS AND OXIDATIVE STRESS IN PROSTATE CANCER-A MECHANISTIC SUPPORT FOR PREVENTION STRATEGIES

Lin H, Bryskin I, Laflamme P, Kleinmann N, Lu JP, Wilson B, Singh G, Pinthus JH

INTRODUCTION: Epidemiological evidences suggest that dietary fat play a role in the etiology of prostate cancer (PC). Oxidation of fatty acids (FA) results in the generation of reactive oxygen species (ROS) which have been postulated to play a key role in the initiation and progression of PC. We hypothesized that androgens, which stimulate the growth of PC, also play a role in FA uptake and degradation and consequently increase ROS production.

METHODS: The model used compared the effect of a synthetic androgen (R1881) and an androgen receptor (AR) blocker (bicalutamide) on androgen-sensitive LNCaP and androgen responsive 22rv1 cells versus that on the androgen- independent CL1 subline that was derived from the parental androgen-sensitive LNCaP cells. Methods used were immunofluorescence, confocal microscopy, western blot, flow cytometry, 3H-oleate uptake and C14 radiolabeled long chain FA degradation studies.

RESULTS: Androgen supplementation (AS) increased the expression of the plasma membrane fatty acid binding protein (FABPpm) leading to increase uptake of fluorescently labeled FA and of 3H-oleate only in PC cells that express the AR. Bicalutamide inhibited this phenomenon. AS significantly increased the oxidation of FA by increasing the levels of CPT1, the rate limiting enzyme in this pathway. Subsequently we demonstrated that blockage of mitochondrial ROS generation by 2 different inhibitors- rotenone and TTFE could eliminate the androgen induced generation of cellular ROS which is coupled to FA oxidation, bringing it to the same levels of PC cells that were deprived of androgens or treated with bicalutamide.

CONCLUSIONS: The uptake of FA into PC cells is androgen regulated through the increased expression of FABPpm. More FA are therefore available for mitochondrial oxidation, a process that is also positively regulated by androgens, leading to increased production of ROS that are associated with cancer cell proliferation and mutagenesis. These results may support the rationale for PC prevention using 5-alpha reductase inhibitors, dietary restrictions or anti-oxidants, all of which have different inhibitory but complementary effect on the proposed mechanism.

PHOTODYNAMIC THERAPY FOR UROLOGICAL MALIGNANCIES: PAST TO CURRENT APPROACHES

Pinthus JH, Bogaards A, Weersink R, Wilson BC, Trachtenburg J

PURPOSE: Modern PDT for urological tumors is a potentially selective approach in which in situ photosensitization by a nontoxic drug, locally activated by light, generates cytotoxic reactive oxygen species, causing cell death. While urological clinical experience with PDT is largely limited to treatment for superficial bladder cancer, the advent of novel photosensitizers and technologies for treatment planning, light delivery and dosimetry, PDT for prostate and other urological cancers appears increasingly realistic.

MATERIALS AND METHODS: We reviewed the current literature on PDT for urological tumors, in addition to recent emerging data from our laboratory and elsewhere.

RESULTS: Remarkable progress has been made in the field of photochemistry and photobiology. Together with improved optical delivery and imaging systems PDT holds promise as an alternative, minimally invasive and potentially curative treatment for localized solid tumors as well as for palliative treatment for isolated, clinically problematic metastases.

CONCLUSIONS: Current experience with photodynamic therapy using contemporary photosensitizing agents and light sources is mainly restricted to in vivo experimental models and early phase clinical trials. However, ongoing preclinical work and clinical trials indicate that safer and effective PDT treatments in uro-oncology are imminent.

PROSTATE CANCERS SCORED AS GLEASON 6 IN PROSTATE BIOPSY ARE FREQUENTLY GLEASON 7 TUMORS AT RADICAL PROSTATECTOMY: IMPLICATION ON OUTCOME

Pinthus JH, Witkos M, Fleshner NE, Sweet J, Evans A, Jewett A, Krahn MA, Alibhai S, Trachtenberg J

PURPOSE: Differentiation between Gleason score 6 and 7 in prostate biopsy is important for treatment decision making. Nevertheless, under grading errors compared with the actual pathological grade at radical prostatectomy are common. We compared the characteristics and outcomes of tumors that were scored 6 on prostate biopsy but were 7 on subsequent radical prostatectomy pathological evaluation to those in tumors with a consistent rating of Gleason score 6 or 7 at biopsy and surgery.

MATERIALS AND METHODS: We performed a retrospective database analysis from our referral center (1989 to 2004). We compared pre-prostatectomy characteristics, radical prostatectomy pathological features and the post-radical prostatectomy prostate specific antigen failure rate, defined as any 2 consecutive detectable prostate specific antigen measurements, in 3 subgroups of patients, including 156 with matched Gleason score 6 in the prostate biopsy and radical prostatectomy, 205 with upgraded Gleason score 6/7, that is prostate biopsy Gleason score 6 and radical prostatectomy Gleason score 7, and 412 with matched Gleason score 7 in the prostate biopsy and radical prostatectomy.

RESULTS: Radical prostatectomy Gleason score matched the prostate biopsy score in 38.2% of biopsy Gleason score 6 and 81.4% of biopsy Gleason score 7 cases. Higher prostate specific antigen was associated and an increased percent of cancer in the prostate biopsy was predictive of discordance between the prostate biopsy and radical prostatectomy Gleason scores ($p < 0.001$). Margin ($p = 0.0075$) or seminal vesicle involvement ($p = 0.0002$), cancer volume ($p < 0.001$) and the prostate specific antigen failures rate ($p = 0.014$) were significantly higher in under graded Gleason score 7 cancer compared to those in matched Gleason score 6 cases. However, they were comparable to those with a matched Gleason score 7 tumor grade ($p = 0.66$).

CONCLUSIONS: Almost half of tumors graded Gleason score 6 at biopsy are Gleason score 7 at surgery. Upgraded Gleason score 6 to 7 tumors have outcomes similar to those of genuine Gleason score 7 cancer. For prostate biopsy Gleason score 6 tumors clinicians should consider the overall likelihood of tumor upgrading as well as specific patient characteristics, such as prostate specific antigen and the percent of tumor in the prostate biopsy, when contemplating treatments that are optimized for low grade tumors, including watchful waiting or brachytherapy.

PLASMA CHROMOGRANIN-A (CGA – A POTENTIAL MARKER FOR DIAGNOSIS, MONITORING AND MANAGEMENT OF PROSTATE CANCER PATIENTS

Leibovitch I, Pinthus JH, Sella BA, Ramon J

INTRODUCTION: Neuroendocrine differentiation of prostatic adenocarcinoma is a recognized phenomenon, which is believed to parallel tumor progression to hormone refractory state. Circulating CgA levels were shown to reflect neuroendocrine differentiation and were found to correlate with the stage and the state of hormone refractoriness. Hence, CgA may become a marker for diagnosis, monitoring and management of prostate cancer patients.

PATIENTS AND METHODS: CgA level was measured in plasma samples which were obtained from 40 patients with prostate cancer, using the ELISA kit (DAKO, Glostrup-Denmark). The normal range of CgA was 2-18, SD = 4. The normal threshold was hence set to 26 u/L calculated as upper normal level + 2SD. Additionally, serum levels of PSA, CEA, CA-125, CA-15.3 and CA-19.9 were measured at that time. Clinical data was collected from medical records.

RESULTS: Overall, CgA was elevated in 18 patients (45%) including 25% of the patients with organ confined disease, 52.9% with locally advanced disease, 71.4% of the patients with metastases, 75% of the patients with hormone refractory prostate cancer and 23.1% of patients with hormone sensitive disease ($p = 0.009$). Mean CgA and PSA levels among patients with elevated CgA was 100.2 u/L (27-717) and 301 ng/ml (4.5-1450) respectively. In comparison to 18.8 u/L (14-26) and 14.7 ng/ml (2.6-59.7) respectively, in patients with CgA within the normal range ($p < 0.05$). PSA at the time of CgA sampling did not differ among the two groups.

CONCLUSIONS: In this study high plasma CgA levels correlated with known poor prognostic factors including advanced and metastatic disease at the time of presentation, high pretreatment PSA levels and hormone refractoriness. CgA levels which reflect neuroendocrine differentiation of prostatic carcinoma may have a diagnostic, therapeutic and prognostic role in the management of prostate cancer patients.

ANDROGEN DEPENDENT REGULATION OF MEDIUM AND LONG CHAIN FATTY ACIDS UPTAKE IN PROSTATE CANCER

Pinthus JH, Lu JP, Bidaisee LA, Lin H, Gupta RS, Singh G

BACKGROUND: Epidemiological and experimental studies suggest that both fatty acids and androgens have a role in the development and progression of prostate cancer (PC). Plasma membrane fatty acid binding protein (FABP(pm)) is a transporter of medium and long chain fatty acids (MCFA and LCFA) across the plasma membrane, and is identical to the mitochondrial protein aspartate aminotransferase (mAAT) that is regulated by testosterone only in prostate epithelial cells, a site where PC initially develops. We therefore hypothesized that FABP(pm) is also regulated by androgens.

METHODS: We examined the effect of a synthetic androgen, R1881, and that of androgen receptor (AR) blocker, bicalutamide, on the expression of FABP(pm) and mAAT and on the uptake of fatty acids in the androgen-sensitive LNCaP, androgen responsive 22rv1 and androgen-independent CL1 human PC cells. This was done using immunofluorescence and confocal microscopy, Western blot, flow cytometry, and (3)H-oleate uptake studies.

RESULTS: Androgen supplementation increased the cellular and surface expression of FABP(pm) and mAAT and increased the uptake of fluorescently labeled MCFA and LCFA and that of (3)H-oleate only in PC cells that express the AR. Bicalutamide inhibited this phenomenon.

CONCLUSIONS: The uptake of MCFA and LCFA into PC cells is androgen regulated as well as the expression of FABP(pm) and mAAT.

ATM ACTIVATION IS ACCOMPANIED WITH EARLIER STAGES OF PROSTATE TUMORIGENESIS

Fan C, Quan R, Feng X, Gillis A, He L, Matsumoto ED, Salama S, Cutz JC, Kapoor A, Tang D

ABSTRACT: The ATM (ataxia telangiectasia mutated) kinase plays an essential role in maintaining genome integrity by coordinating cell cycle arrest, apoptosis, and DNA damage repair. Phosphorylation of ATM at serine 1981 (ATMpSer1981) by DNA damage activates ATM, which subsequently phosphorylates H2AX Ser139 (gammaH2AX), Chk2 Thr68 (Chk2pThr68), and p53 Ser15 (p53pSer15). To determine the role of the ATM pathway in prostate cancer tumorigenesis, we have analyzed 35 primary prostate cancer specimens for ATMpSer1981 (ATM activation), Chk2pThr68, gammaH2AX, and p53pSer15 by immunohistochemistry (IHC) in normal glands, prostatic intraepithelial neoplasias (PINs), and carcinomas. Increases in the intensities of ATMpSer1981, Chk2pThr68, and gammaH2AX and in the percentage of cells that are positive for ATMpSer1981, Chk2pThr68, or gammaH2AX were observed in PINs ($p < 0.001$) compared to normal prostatic glands and carcinoma. However, this pattern of immunostaining was not seen for p53pSer15. Thus, ATM and Chk2 are specifically activated in PINs. As PINs are generally regarded as precursors of prostatic carcinoma, our results suggest that ATM and Chk2 activation at earlier stages of prostate tumorigenesis suppresses tumor progression, with attenuation of ATM activation leading to cancer progression.

DUAL EXPRESSION RECOMBINASE BASED (DERB) SINGLE VECTOR SYSTEM FOR HIGH THROUGHPUT SCREENING AND VERIFICATION OF PROTEIN INTERACTION IN LIVING CELLS

Lu JP, Beatty L, Pinthus JH

ABSTRACT: Identification of novel protein interactions and their mediators is fundamental in understanding cellular processes and is necessary for protein-targeted therapy. Evidently high throughput formatting of these applications in living cells would be beneficial, however no adequate system exists. We present a novel platform technology for the high throughput screening and verification of protein interactions in living cells. The platform's series of Dual Expression Recombinase Based (DERB) destiny vectors individually encode two sets of recombinase recognizable sequences for inserting the protein open reading frame (ORF) of interest, two sets of promoters and reporter tags in frame with the ORFs for detecting interactions. Introduction into living cells (prokaryotic and eukaryotic) enables the detection of protein interactions by fluorescence resonance energy transfer (FRET) or bimolecular fluorescence complementation (BiFC). The DERB platform shows advantages over current commercialized systems by introducing recombinase based cloning and compatible accepting vectors validated through proof-of-principle experiments and the identification of an unknown interaction.

CARDIOVASCULAR EFFECTS OF ANDROGEN DEPLETION AND ANDROGEN REPLACEMENT THERAPY

Pinthus JH, Trachtenberg J, Klotz L

**IDENTIFICATION OF THE INDIVIDUAL'S INHERENT SENSITIVITY TO OXIDATIVE STRESS: TOWARDS
BETTER TRIAGE OF LOCALIZED PROSTATE CANCER PATIENTS FOR RADIATION**

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