

Extremely Early Diagnostic Test for Prostate Cancer

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ABSTRACT

Purpose: This article reports the results of a blinded fibre diffraction study of skin samples taken from TRAMP mice and age-matched controls to determine whether changes noted in fibre diffraction studies of human skin were present in these TRAMP mice studies. These mice are bred to progress to Gleason Type 3 to Type 5 prostate cancer. **Methods:** Small strips, 1 mm × 5 mm, cut from the mouse skin samples were loaded into cells in the same way as human samples and slightly stretched to remove the crimp. They remained fully hydrated throughout exposure to the synchrotron beam. **Results:** The added change that was reported for prostate cancer in 2009 was obtained for all TRAMP mice samples, indicating that this change can be read as High Grade Cancer in human diagnostic tests. **Discussion:** These changes were evident for all 3 and 7 week old TRAMP mice samples but not for any of the control samples. This indicates that the changes in the fibre diffraction patterns appear much earlier than in any other available prostate cancer diagnostic test, as none of these can verify the presence of prostate cancer in the TRAMP mice before 10 weeks of age. The fibre diffraction test is therefore the most accurate and earliest test for high grade prostate cancer.

Keywords: Prostate Cancer, Early Diagnostic Test, Murine Study

1. Introduction

Prostate cancer is the most commonly occurring cancer worldwide in men over 50 years of age. At this time it is responsible for more deaths in Australia and in the USA than deaths in women from breast cancer. At present the investigative process for diagnosing prostate problems involves serial PSA (prostate specific antigen) readings, ultrasound testing, and prostate biopsies which are particularly invasive procedures requiring general anaesthetic, and up to 20 or sometimes 30 core biopsies of the prostate. The risk of infection following these procedures is significant and the postoperative recovery takes a variable amount of time depending on other co-existent patient demographic factors. The literature and research to date has spent most of its energy investigating ways in which the PSA levels are analyzed as well as the risk factors which seem to be linked to lifestyle factors, ethnicity, demographic factors and diet. The rate of doubling of the PSA levels in the blood is one factor which the specialists use to determine the degree of severity of the prostate cancer, the rate of progress of the disease and it is used as a predictor of the probability of the prostate cancer being one type or the other. It is an imprecise

measurement however which is influenced by a number of other variables and the patient must wait for an interval of time to elapse in order to determine the nature of the possible type of prostate cancer. Studies quote that 230,000 new cases of prostate cancer were expected in the USA alone in the year 2005 [1]. Prostate cancer is now responsible for more deaths in men in Australia than deaths in women from breast cancer.

The highly accurate diagnostic test for prostate cancer using low angle fibre diffraction of human skin [2,3], (FDD) would appear to offer a greatly improved early detection of prostate cancer. In previous studies, 296 very small skin samples, (either 3 mm full skin biopsies taken from anywhere on the body or small 1 mm × 5 mm tissue sections from the edge of samples removed in operations), were included in sets of skin samples taken from persons with a wide range of other cancers, other diseases, and from healthy individuals. These skin studies included but were not limited to persons with diabetes mellitus, myxomatous heart valves and cancers of the breast, lung, prostate and skin and some of these have been reported [2-8]. Samples from 13 patients with prostate cancer were included amongst these blinded samples. The specific change in the fibre diffraction patterns of skin, obtained

for persons with prostate cancer, was found for all persons known to have prostate cancer and also for 2 persons subsequently proven to have it after X-ray diffraction results were returned. If a patient is suffering from more than one cancer, for cancer types for which specific FDD changes have been confirmed, as in the case of a patient suffering from both melanoma and prostate cancer [3], both changes are evident in the resulting diffraction patterns.

2. Method

This blinded FDD murine study also used small $1\text{ mm} \times 5\text{ mm}$ tissue sections from the edge of the skin samples supplied by The Jackson Laboratory [9] specifically for this study which was carried out at the Advanced Photon Source, BioCAT beam-line Argonne USA. Two skin samples were supplied for each murine skin sample, one from the back and one from the lower body. Two murine sample sets were supplied for each of the 3 and 7 week old TRAMP mice (transgenic adeno-carcinoma of mouse prostate) and for each of their age matched non-carrier mice controls. These 32 samples were placed in phosphate-buffered saline immediately after being surgically removed at the Jackson Laboratory and then shipped and stored at -20° Celsius until required.

For all FDD studies of skin, human and animal, sections of collagenous tissue are carefully removed from the dermal layer of the skin. These samples are placed in a cell specially designed to allow the sample to be stretched slightly to remove the natural crimp and also to maintain 100% humidity during the exposure to the finely focussed X-ray beam [3]. The BioCAT beam-line at The Advanced Photon Source, the third generation synchrotron at the Argonne National Laboratory, USA, was used for this study. Synchrotron X-radiation has many advantages such as vastly increased flux and brilliance. It also offers a choice of a specific wavelength for any experiment; the latter is simply selected using a monochromator. For this study, a finely focussed X-ray beam, of energy 12 KeV was passed completely through the sample. Exposure times for the samples in this study were between 1 and 3 seconds. After background removal, the pattern arising from diffracting this finely focussed X-ray beam ($0.1 \times 0.4\text{ mm}$) off the electron density distribution in the 3 week-old mouse samples, **Figure 1**, was recorded on both a detector and on Fuji-Bas imaging plates.

3. Results

TRAMP mice are bred to progress to high grade prostate cancer, *i.e.* Gleason 3 to Gleason 5, but the tumours are not detectable by any other laboratory tests before 10 weeks of age. Our studies show these changes at a much earlier stage in these mice and may suggest the ability to

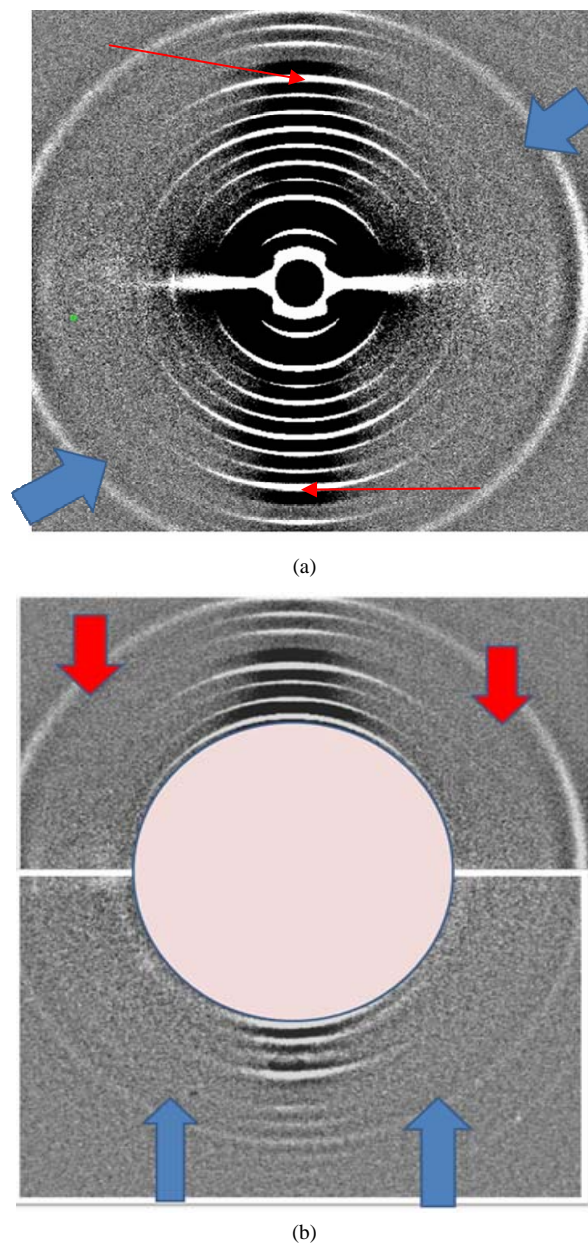


Figure 1. (a) Diffraction pattern of skin taken from a 3 week-old mouse. The blue arrows show the weak diffuse ring associated with prostate cancer. The thin red arrows indicate the 12th order of the collagen meridional pattern. (b) Top half shows weak ring in Tramp mouse results, indicated by red arrows. Bottom half from 3 or 7 week-old controls shows no such rings as indicated by blue arrows.

detect specific rings patterns well before clinical or biochemical parameters become positive for prostate cancer. Since the 16 Tramp mice all gave a diffuse ring or arc between the 13th and 14th orders of the normal skin FDD patterns as did all human prostatectomy samples, it is reasonable to assume the origin of this diffuse ring is Gleason Type 4-6. No such arcs or rings were evident in any of the

control mice.

4. Discussion and Conclusions

The stand out message when it comes to detecting the presence of prostate cancer is early detection of the disease. It is important that the patient is given an accurate prediction of the aggressive nature of the disease, as described by the two forms of the disease. At present, however, early detection is not always possible or 100% reliable due to the limitation of the investigative processes currently available. Being able to detect the disease early and to predict the nature and the type of the prostate disease has huge ramifications when it comes to recommendations from medical staff and decisions to be made by the patient regarding the form of treatment which may be offered and accepted in light of the possibly post operative risks for infection, impotence, and incontinence and for the ultimate survival of the patient.

This paper uses a murine study to indicate that FDD can accurately determine the presence of prostate cancer long before any other test in use. It also offers a clear indication of which pattern change is related to the high risk form of this cancer, *i.e.* Gleason Types 3-5. A subsequent study of 26 samples from Whittington Hospital verified these results for high grade prostate cancers and indicated a different change for low grade type tumours and, after checking, this study will be reported in a subsequent publication.

These studies verified that this highly accurate, very robust fibre diffraction test is useful for diagnosing the presence of prostate cancer from the very beginning of its growth and earlier than any other method currently available. It also confirms that a diffuse ring indicates Gleason Type 3-5 prostate cancers are present. The fact that high risk prostate cancers can be determined by this test would eliminate the necessity for surgical removal of low grade types of this cancer together with the possible side-effects.

Fibre diffraction diagnostic tests may therefore not only lead to a replacement of the imprecise and invasive nature of establishing a diagnosis by prostate biopsy but it could also replace the prostate specific antigen (PSA) test. Fibre diffraction tests use one very small 3 mm skin biopsy, which can be taken from any loose skin on the body, possibly needing one small stitch. It is much less invasive than present tests and at the same time eliminates the possibility of infections. FDD could therefore inexpensively provide the much needed, very accurate and relatively non-invasive standard screening test for prostate cancer needed to reduce the current very high death-rate for this cancer. Other mice are being bred to check for lower Gleason grades of prostate cancer and further human studies are in progress. Clinical trials are

being planned for 2011.

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