Novel human prostate cell lines derived from the transition and peripheral zones of the prostate for carcinogenesis studies

JENNIFER WEAVER[1], CECILIE BRISCOE[1], SLAWOMIR KATA[2], CHRIS GOODMAN[2], HORST ZITZELSBERGER[3], LUDWIG HIEBER[3] and ANDREW RICHES[1]

[1]School of Medicine, University of St. Andrews, St. Andrews; [2]Department of Urology, NHS Tayside University Hospitals, Dundee, Scotland, UK; [3]Institute of Molecular Radiation Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

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Abstract. Epithelial cell lines were established from the transition and peripheral zones of human prostate by transduction with cdk4 and hTERT. The properties of these lines were investigated using immunocytochemical markers, ability to generate anchorage-independent colonies and by spectral karyotyping (SKY). Cells were exposed to fractionated doses of gamma irradiation to investigate their ability to transform. Cell lines were established from the transition and peripheral zones of human prostate. The expression of CD133, CK5, CK14, CK18, p16, PSCA, p63 and c-myc varied between the lines from the two regions. The line derived from the peripheral zone exhibited properties of a tumour line. A similar pattern was observed in two separate transductions. It was thus unlikely to be an *in vitro* transformation event, which is very rarely observed with human cells *in vitro*, and thus more likely to be derived from the immortalisation of a quiescent tumour clone. Fractionated irradiation of the transition zone cell line resulted in forming of transformed colonies. The transformed and tumour line had marked chromosomal rearrangements as demonstrated by SKY analysis. Cell lines have been derived from different zones of human prostate for studies on radiation carcinogenesis. The unirradiated cell line derived from the peripheral zone exhibited chromosomal rearrangements similar to those observed in prostate carcinoma. The cell line derived from the transitional zone exhibited a near diploid karyotype and could be transformed following exposure to fractionated doses of gamma irradiation.

Correspondence to: Professor Andrew Riches, Medical and Biological Sciences Building, School of Medicine, University of St. Andrews, St. Andrews KY16 9TF, Scotland, UK
E-mail: acr1@st-andrews.ac.uk

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Introduction

Human prostate cells have been studied *in vitro* and there are well established prostate carcinoma cell lines: DU145, LNCaP and PC-3 (1). These cell lines were not derived from a primary tumour but from metastatic lesions.

Cell lines have also been created by immortalising prostate cells with viral constructs (2-8). While this proved useful for deriving cell lines, they have major disadvantages. For example immortalization with the SV40 genome results in expression of the viral genome and perturbs the p53 and Rb pathways which leads to cell instability (4).

Rhim *et al* (9) transfected prostate epithelial cells with the entire HPV-18 genome, which resulted in immortal cells that did not undergo malignant conversion. However, introduction of the Kirsten murine sarcoma virus or the activated Ki-*ras* oncogene led to the malignant transformation of these cells. Other groups have also used the HPV-18 viral genome (10-12). Later groups looked at immortalisation with the HPV-16 E6 and/or E7 viral genes (1,13-15). Use of the E6 and E7 genes together resulted in cells with an increased proliferative index so E6 was used alone which had the dual effect of inactivating p53 and activating telomerase which extended the lifespan of the cultured prostate cells (15). Human prostate epithelial cells have also been immortalised by insertion of one non-viral gene only, *c-myc* (16). These cells showed many normal cell characteristics, such as an intact p53 response and a senescence-like growth arrest in response to the *Ras* gene, but the cells transformed in culture without any additional genetic input (16).

Yasunaga *et al* (17) immortalised a human prostate epithelial cell line from a patient with early onset prostate carcinoma by overexpressing the *hTERT* gene. Gu *et al* (18) utilised this cell line RC-58T/hTERT and characterised it. He further created a clonal line called RC-58T/hTERT SA#4, and human prostate epithelial cell lines with the overexpression of *hTERT* with cells from tissue taken from benign and malignant sections of a tumour (19). However, these cell lines all had multiple chromosomal aberrations (19). Kogan *et al* (20) successfully immortalised human prostate epithelial and stromal cells with telomerase and found that the cell lines expressed a normal p53 pathway. Miki *et al* (21) looked at the

cell lines created by Gu *et al* (19) and further characterised them and demonstrated that they expressed the stem cell markers, CD133 and CXCR4. Shao *et al* (22) immortalised primary human prostate epithelial cells with hTERT and demonstrated this required downregulation of $p16^{INK4a}$. Immortalised human prostate cell lines have also been utilised for radiation carcinogenesis studies (23).

The insertion of cdk4 and hTERT has successfully been used to immortalise human bronchial epithelial cells (24). This cell line was immortalised without the presence of viral oncogenes and did not form anchorage-independent colonies in soft agar or form tumours in nude mice (24). These cells had an intact p53 checkpoint and are a stable model. This study reports establishment of cell lines using these two constructs. Prostate epithelial cells from different zones of human prostate were transduced with these constructs and utilised for studies on radiation carcinogenesis.

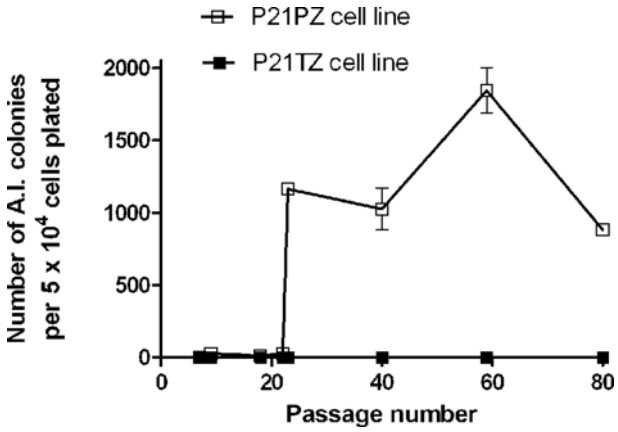

Figure 1. Anchorage-independent colony formation at different passage number of two human prostate immortalised cell lines derived from the transition zone of the prostate (P21TZ) and the peripheral zone of the prostate (P21PZ) from the same patient.

Materials and methods

Establishment of cell lines from prostate. Cell lines were developed from tissue chippings collected during elective transurethral resection of the prostate gland from patients with no suspicion of prostate cancer (with full ethical approval, LREC 191/01). TUR chips were collected first from the transition zone (origin of <20% of prostate cancers) and then from the peripheral zone (containing 70% of the prostate glandular elements and the origin of almost 80% of prostate cancers) (25,26). The TUR chips were flushed out sequentially so that specific regions of the prostate could be sampled. The pathology report indicated the presence of fibromuscular and glandular hyperplasia in the TUR chips but no evidence of malignancy in any of the chips sampled although occasional atrophic glands and focal inflammation were present. The TUR chips were minced into 1-mm cubes and treated with collagenase Type 1 (Sigma C0130, 4000 U/ml) and epithelial cells were cultured in Prostate Epithelial Cell Basal Medium with additives (PrEBM, Lonza). Prostate epithelial cells were transduced with the *cdk4* gene and then subsequently with the *hTERT* gene, after establishment of primary cultures as reported before using supernatants from ψ-CRIPwtcdk4LXSNneo cells followed by *hTERT* using supernatants from ψ-CRIP pBABEpurohTERT cells (24).

Epithelial outgrowths were established from the prostate samples following collagenase treatment. Following 2 passages to expand the cell numbers, cells were transduced with the cdk4 construct and selected for antibiotic resistance (G418) and following a further passage transduced with the hTERT construct with selection with puromycin. The cells were then further passaged and screened at appropriate passage numbers for anchorage-independent growth.

Cell lines were created from the same patient from the transition zone were denoted by the letter TZ and from the peripheral zone denoted the letter PZ.

Epithelial cell culture. Prostate epithelial cells were cultured in PREBM with additives. Cloned cell lines were also derived from the early passage line derived from the peripheral zone (before passage 9) by plating the cells at low density and using cloning rings.

Anchorage-independent colonies in agar. The cells were screened for anchorage-independent growth in agar (27). Briefly a base layer of 5% agar and medium was added to 60-mm round dishes. The top layer consisted of 3% agar medium and cells (50,000 cells/dish). The dishes once set are placed in the incubator at 37˚C in a humidified atmosphere of 5% CO_2 and left for 3 weeks. If colonies are present, half of the plates were stained with INT and colonies on the unstained plates were picked and resuspended in a 12-well tissue culture plate in 1 ml PREBM medium and incubated for 1-2 weeks until epithelial cells were seen to grow outwards from the colony to establish cloned cell lines.

Immunocytochemistry. Cytospin preparations of epithelial cells were fixed on slides in 100% ice-cold acetone. Slides were treated with a protein block and the ICC protocol was followed according to the manufacturer's instructions (Dako). Antibodies were diluted according to the suppliers recommendations [CK14, CK5, CK18, Telomerase, p63, p16, and c-Myc (NovoCastra); PSCA and CD133 (Abcam)].

Secondary antibody DAB biotinylated link solution and DAB chromogen was used to visualise the results. The slides were counterstained with haematoxylin.

SKY analysis. SKY analysis on metaphase chromosome spreads was carried out as reported previously (28).

Invasion assay. To determine if the cells were invasive they were applied to a Matrigel™ porous membrane (BD Biocoat™ Matrigel Invasion Chamber 24-well plate 8.0 μm, 354480) (29) PREBM medium containing the additives was used as a chemoattractant. The protocol described in the manufacturer's instructions was followed. Invasion was calculated as a percentage of the average number of invading cells through the Matrigel membrane over the average number of invading cells through a control insert.

Radiation transformation. Cells were irradiated with a gamma source (IBL 437C) using Caesium 137, at a dose rate

Table I. Immunocytochemical analysis of cells from P21TZ, P21PZ, P21PZ (clone-a) and P21TZ 40Gy (clone-a) cell lines tabulating the expression of CD133, CK5, p63, p16, CK14, telomerase, PSCA, CK18 and c-myc.

	P21TZ	P21PZ	P21PZ (clone a)	P21TZ 40 Gy (clone a)
CD133	N	N	N	-
PSCA	Y	Y	Y	Y
CK5	Y	Y	Y	Y
CK14	Y	Y	Y	Y
CK18	N	Y	Y	Y
p16	Y	N	N	N
p63	Y	N	N	Y
Telomerase	Y	Y	Y	Y
c-Myc	N	Y	Y	Y

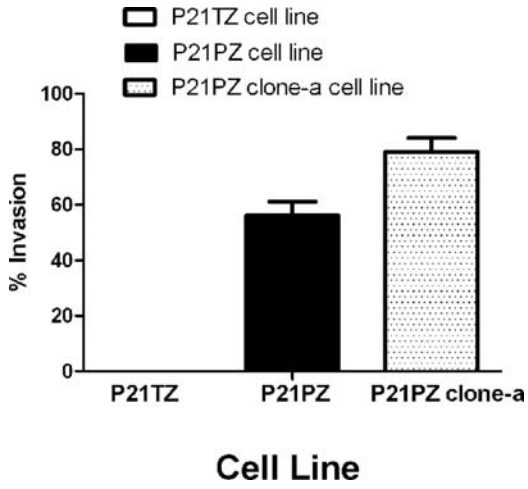

Figure 2. Assessment of the invasive properties of the human prostate cell lines using a Matrigel porous membrane assay.

of 3.64 Gy/minute. Cell lines were exposed to fractionated doses of 2 Gy (27).

Results

Anchorage-independent colony formation. The P21TZ cell line, derived from the transition zone of the prostate, did not produce any anchorage-independent colonies at the passage numbers studied (up to passage number 80). The cell line P21PZ, derived from the peripheral zone of the prostate, initially showed no colony formation up to passage 10 and then a few colonies up to passage 20 followed by a marked increase in colony number from passage 20 onwards (Fig. 1). This pattern was repeated in two independent transduction experiments. An anchorage-independent colony was selected and grown up and designated P21PZ (clone-a).

Transformation studies. Following exposure of the P21TZ cell line to fractionated doses of 2 Gy gamma radiation, no anchorage-independent colonies were observed in the irradiated and unirradiated cultures until an accumulated dose of 40 Gy where (4.0±0.5) colonies per 10^5 cells were observed. No colonies were observed at any passage number with the unirradiated P21TZ cells. A cloned cell line [P21TZ 40 Gy (clone-a)] was selected from the irradiated cultures. This cloned cell line produced a marked increase in anchorage-independent colonies from (4.0±0.5) per 10^5 cells to (1150±0.5) per 10^5 cells.

Immunocytochemical studies. The cell lines, P21TZ, P21PZ, and P21PZ (clone-a) all stained positive for PSCA (Table I). P21PZ and P21PZ (clone-a) stained positive for *c-Myc* whereas P21TZ was negative for *c-Myc* which is typically up regulated in tumourigenic cells (16). All cell lines are positive for CK5 and CK14 which are basal cell markers indicating that the cells are of basal origin.

P21PZ and P21PZ (clone-a) were positive for CK18 whereas P21TZ was negative. All cell lines are positive for telomerase expression as would be expected as the telomerase gene has been activated so that the cells are immortal. P21TZ is positive for p63 expression but the P21PZ and (clone-a) are negative. The gene p63 encodes multiple isotypes that transactivate p53 reporter genes and induce apoptosis. P21TZ is positive for p16 protein and P21PZ and P21PZ (clone-a) are negative for p16 protein staining. The gene p16 is a tumour suppressor gene and if cells are stressed it binds to cyclin dependent kinase 4 and prevents binding of cyclin D1 and the cell goes into apoptosis.

In vitro invasion studies. Cells from the P21TZ cell line did not invade through the Matrigel insert (Fig. 2) whereas cells from the P21PZ and P21PZ (clone-a) cell lines showed marked invasion (56% and 79%).

Spectral karyotyping (SKY). The P21TZ cell line is near-diploid with a trisomy 20 which is often seen in immortalised prostate cell lines (karyotype: 47,XY,+20) (Fig. 3A). Following exposure to fractionated doses of gamma irradiation a transformed clone was selected. The main karyotype of P21TZ 40 Gy (clone-a) is 49,XY,+5,+9,+20,der(2(del(2)(p11.1,der(7)t(15;7;2),der(15)t(7;15),der(21)t(7;21) (Fig. 3B).

There is a subclone with the karyotype: 49,XY,+5,+8,+9,+20,der(2(del(2)(p11.1,der(7)t(15;7;2),der(15)t(7;15). This clone has in addition a trisomy 8 and is missing the der(21)t7;21).

The cell line derived from the peripheral zone exhibited abnormalities already and thus could not be used for transformation studies. The P21PZ and P21PZ (clone-a) cell lines show near-tetraploid karyotypes with modal chromosome numbers of 75-90 (Fig. 3C) and 75-82 (Fig. 3D), respectively. Both of the cell lines have lost their Y chromosome. About half of the cells of P21PZ cell line were karyotyped as: 75-90, XX,der(10)t(10;8)x2,der(11)t(11;15),der(17)t(17;20)x2, der(20)t(20;17)x2. There were three further clones with additional chromosome rearrangements such as der(15)t(15;17), der(7)t(7;5) or der(8)t(8;5) present in this culture.

The P21PZ (clone-a) showed an even more complex karyotype of 75-82,XX,i(X)(q10),add(1)(p33→pter),ace(2;5),

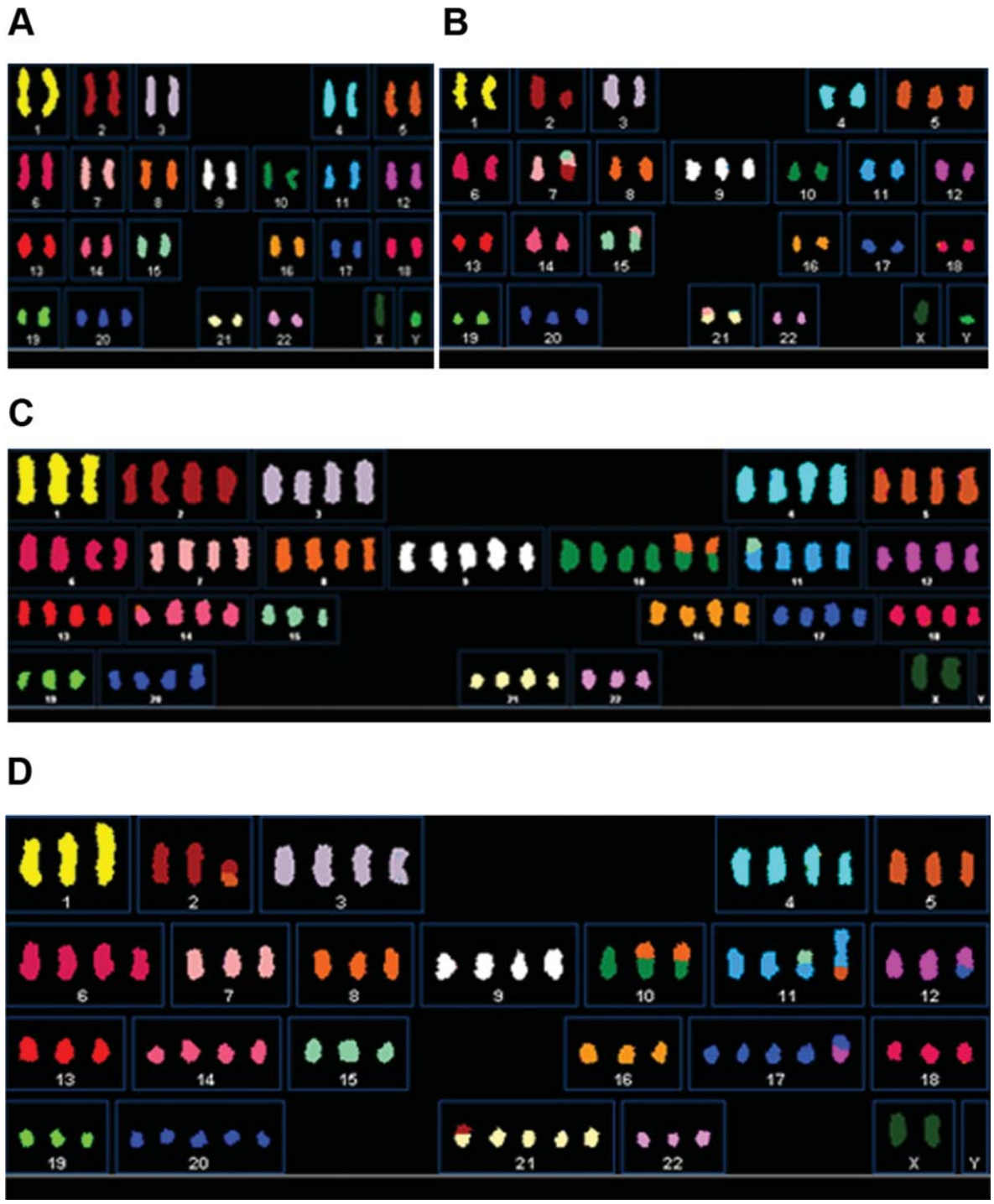

Figure 3. Spectral karyotyping (SKY) of prostate cell lines. Cytogenetic analysis demonstrated by classified image in pseudocolours. (A) Cytogenetic analysis of the P21TZ cell line identified a normal chromosome karyotype except for three copies of chromosome 20. (B) Structural aberrations in the transformed P21TZ 40Gy (clone-a) cell line identified by spectral karyotyping (SKY). (C) Structural aberrations in the P21PZ cell line identified by spectral karyotyping (SKY). (D) Structural aberrations in the P21PZ (clone-a) cell line identified by spectral karyotyping (SKY).

der(10)t(10;8)x2,der(11)t(11;5),der(11)t(11;15),der(12)t(12;17),der(17)t(20;17;12),der(17)t(17;20),der(20)t(20;17)x2, der(21)t(21;2). The additional material in one of the copies of chromosome 1 was found to be chromosome 1 specific. Besides this main clone in P21PZ (clone-a) there were also three subclones with der(2)t(2;10) or der(7)t(7;17) or the derivative chromosome 21 was der(21)t(21;7) instead of der(21)t(21;2).

Early passage P21PZ plastic derived cloned cell lines. As the early passage cell line from the peripheral zone was likely to be a mixed population of normal and transformed cells, cloned cell lines were derived from the early passage transduced cells at a stage before anchorage-independent colonies were observed. All seven cloned cell lines expressed anchorage-independent growth, were positive for CK18 and CK5 expression, had undetectable levels of c-Myc, exhibited

Table II. Properties of seven P21PZ plastic cloned cell lines derived from an early passage culture of P21PZ (before passage 9): characterisation by anchorage-independent colony formation, immunocytochemical analysis for cytokeratin 18 and 5, invasion assay and number of chromosomes per cell in metaphase.

P21PZ cloned cell lines	No. A I colonies/ 100,000 cells plated	ICC c-Myc	CK18 and CK5	% Invasion	No. chromosomes/ cell (metaphase spread)
A	1.5±0.5	Negative	Positive	3.8	65-74
B	20±0.5	Negative	Positive	2.7	65-74
C	11±0.5	Negative	Positive	1.3	54-79
D	27±0.5	Negative	Positive	3.5	50-79
E	20±0.5	Negative	Positive	4.5	60-78
F	8±0.5	Negative	Positive	3.7	68-76
G	7±0.5	Negative	Positive	1.1	59-64

limited invasion through a Matrigel porous membrane, and had abnormal chromosome numbers per cell (Table II).

Discussion

The aim of this study was to create and characterise cell lines from different zones of human prostate tissue by inserting and expressing the *cdk4* and *hTERT* genes. Human cell lines were created from the transition and peripheral zones of the prostate. TUR chips were selected by flushing out sequentially so that we could be sure of sampling the transition and peripheral zones. The P21TZ cell line did not form anchorage-independent colonies with increasing passage number, however, the peripheral zone cell line (P21PZ) produced anchorage-independent colonies around passage 10. Independent repeats of the transduction with cdk4 and hTERT with the early passage P21PZ cells gave exactly the same results with anchorage-independent colony formation. Colonies being observed after passage 10 and increasing with further passage thus suggesting that this was not an *in vitro* transformation event. It is likely that an undiagnosed sub-clone of prostate cancer was present in the patient's peripheral zone and this was selectively grown up and immortalised over time. It is unlikely that transduction with cdk4 and hTERT has initiated this change as no other authors have reported spontaneous transformation with a variety of human epithelial cells immortalised using this methodology and we observed the same pattern with independent transductions. It is well established that prostate cancer mostly arises in the peripheral zone and quiescent foci can be frequently detected in autopsy material.

In all aspects there is evidence to show that the P21TZ and P21PZ and P21PZ (clone-a) are very different from each other. The growth and morphology was the first evidence for this. The immunocytochemistry results show that the cells of the lines are expressing basal cell markers CK14 and CK5 so therefore can be assumed are of basal origin from both the transition and peripheral zones. All cell lines were positive for PSCA which is an early cell precursor marker.

P21TZ was negative for the expression of *c-Myc* where as P21PZ and P21PZ (clone-a) exhibited marked positive nuclear staining (Table I). This suggests that P21TZ was a normal cell line and P21PZ and P21PZ (clone-a) were transformed cell lines. P21TZ was also negative for CK18 where as P21PZ and P21PZ (clone-a) were very positive. CK18 is a marker for luminal secretory cells. It is highly expressed in tumourigenic cancer cell lines such as PC3, LNCAP and DU145 (30) and a majority of prostate carcinomas (31). Interestingly, the cell line derived from the transitional zone (P21TZ) did not express CK18 whereas the lines derived from the peripheral zone did. All cell lines are positive for telomerase expression as is expected as insertion of the *hTERT* gene causes transcription and activation of the telomerase enzyme, which immortalises the cells so they can divide indefinitely.

p63 is a member of the p53 gene family and encodes proteins that interact with p53 proteins and is expressed in healthy prostate tissue in basal cells as it is required for normal development of prostate tissue (32). Loss of expression of p63 is recorded in most invasive cancers (33) and is required, along with other genes, for cellular apoptosis in response to DNA damage (34). P21TZ is positive for p63 expression and P21PZ and P21PZ (clone-a) were negative, further supporting the view that P21PZ and P21PZ (clone-a) were transformed cell lines.

The $p16^{INK4a}$ gene is a tumour suppressor and encodes p16 protein in mammalian cells (35). p16 protein is often expressed in cells in culture and indicates a normal RB pathway but expression is lost in many cancers including prostate cancer (36). If $p16^{INK4a}$ gene expression is lost then p16 protein is no longer produced and there is no inhibitory pathway to prevent the cells from entering apoptosis when they are stressed. P21TZ is positive for p16 expression as would be expected as the cells are stressed as growing out with physiological conditions (i.e. 95% air 5% CO_2), but as cdk4 is being overexpressed there are higher levels of cdk4 to bind to cyclin D1 than there is p16 protein binding to cdk4 to inhibit it and cause the cell to go into apoptosis. P21PZ and P21PZ (clone-a) did not express p16 and therefore have lost another checkpoint to control cell division. This is evidence that P21PZ and P21PZ (clone-a) are transformed cell lines.

Matrigel invasion assays are a convenient way of assessing the invasive properties of cell lines without using animal

models. P21TZ was non-invasive whereas P21PZ and P21PZ (clone-a) were invasive (Fig. 2). Positive controls using this assay with the PC3M (metastatic prostate cancer cell line) reported by Collins *et al* (29) gave similar results to our cell lines. This is very good evidence that P21PZ and more so P21PZ (clone-a) exhibit properties of a transformed cell.

The SKY analysis for the P21TZ cell line showed that the cells have an almost normal copy number of chromosomes per cell, except it has 3 copies of chromosome 20 which is apparently very common in immortalised prostate cell lines (37,38). P21PZ and P21PZ (clone-a) have extensive chromosome aberrations including many translocations, with an average of 80 chromosomes per cell and loss of the Y chromosome (Fig. 3). The SKY analysis of the spontaneously transformed cell lines had similar amplification and deletion regions as seen in human prostate carcinoma (39,40).

The irradiated P21TZ cloned cell line [P21TZ 40 Gy (clone-a)] produced anchorage-independent colonies and was positive for c-Myc expression. The SKY analysis of this cell line showed there were extra copies of chromosome 5, 9 and 20 in the dominant clone of P21TZ 40 Gy (clone-a) cell line and an extra copy of 8 in the subclone. As previously mentioned, trisomy 20 is very common in immortalised prostate cell lines. Extra copies of chromosomes have been reported in the literature for prostate tumour cell lines (1,17,23).

If we compare the SKY analysis of the cell line transformed with radiation [P21TZ 40 Gy (clone-a)] and the transformed cell line derived from the putative PIN lesion [P21PZ and P21PZ (clone-a)], we see that there are few similarities between the two (Fig. 3). The only similarity is that both cell lines have extra copies of chromosome 5, 9 and 20. However, P21PZ has multiple copies of every chromosome including two copies of the X chromosome which mimics the RC-58T/hTERT transformed cell line derived from a primary tumour of a prostate cancer patient (17). Prostate carcinoma cell lines PC-3, LNCaP and DU145 all have been shown to exhibit structural alterations of chromosomes 1, 2, 4, 6, 10, 15, and 16 but not 5 or 9 (41).

P21PZ and P21PZ (clone-a) both exhibit losses of the 10p arm (Fig. 3). Other hTERT immortalised prostate cancer cell lines also exhibit losses of the p arm (17) and the loss of 10p has been recorded in prostate carcinomas suggesting there is a possible tumour suppressor gene in this location (39,40). The P21PZ cell line also has a 17p deletion in three copies out of four. The *p53* gene is mapped to chromosome 17p13.1 and has been shown to be mutated in tumours arising in the prostate (42), renal (43), colon, lung, oesophagus, breast, liver, brain, reticuloendothelial tissues, and hemopoietic tissues (44). Numerous studies have evaluated the status of the *p53* alterations in prostate cancer (45). Mutations of the p53 tumour suppressor gene are a frequent genetic event in prostate cancer and can be detected in up to 94% of cases (46), *p53* mutations have been reported to be associated with acquisition of androgen-independence and metastasis in prostate cancer (47).

The P21PZ (clone-a) cell line has a prominent 11p amplicon in one of the copies (Fig. 3). Gain of chromosomal 11p was a frequent aberration in lung adenocarcinoma tumours of male smokers (47). A gene, lactate dehydrogenase C (LDH-C), is located at 11p15.1 and an increase in the average transcript level of *LDH-C* with the gain of 11p was found amplified in cases of lung adenocarcinoma (46). It has been reported that LDH-C is up-regulated alongside increased 11p number in many other cancers including prostate (48). LDH-C codes for an isoform of the lactate dehydrogenase family and is normally only expressed in germ cells (49). LDH-C enzyme catalyses lactate into ATP in the glycolysis pathway and thus LDH-C activation in cancer may provide a metabolic rescue pathway in tumour cells by exploiting lactate for ATP delivery (48).

Fractionated doses of gamma radiation have been utilised to transform a human thyroid epithelial cell line (49), a human retinal pigment epithelial cell line (27) and human prostate epithelial cells (23). All cell lines exhibited neoplastic phenotypes including forming tumours in nude mice and anchorage-independent growth. This demonstrates that cells exposed to fractionated doses of gamma radiation over time can be transformed *in vitro*. The cell line P21TZ 40 Gy (clone-a) exhibited characteristics of becoming transformed after accumulating a dose of 40 Gy with anchorage-independent colony growth in agar and chromosome aberrations (Fig. 3). This supports the conclusion that the cell line has been successfully transformed. This alongside with the parent non-transformed immortal cell line P21TZ gives a good model cell system.

As the P21PZ cell line did not produce any anchorage-independent colonies at early passage, we hypothesised that this could be a mixed population of predominantly normal cells with some transformed clones present. Thus, cloning the cell line at this stage would enable us to select normal cell lines with a high probability. Early passage P21PZ cells were plated at low density on plastic. It was anticipated that several of these plastic derived cloned cell lines would be normal. However, all seven cell lines exhibited anchorage-independent growth and had abnormally large number of chromosomes per cell (Table II). None of the cell lines exhibited significant invasion in the Matrigel assay. Similar results have been reported for cells from benign prostatic hyperplasia (BPH) and the PNT1A cell line also derived from a patient with BPH (29). This suggests that all seven cell lines were abnormal (1,19,50). Interestingly all the cloned cell lines express CK18 which is not expressed in the cell line derived from the transition zone. It can be concluded that the P21PZ cell line did not compose of a mixture of normal and abnormal cells but is likely to be an unstable population of abnormal cells. It thus seems likely that the patient P21 may have had an undiagnosed tumour clone or a prostatic intraepithelial neoplasia (PIN) in the peripheral zone of the prostate and that these cells have been immortalised early on and propagated in culture. PIN lesions in human prostates are very common in men with increasing age and thought to be the precursor of prostate cancer (51-53) many of which do not progress to clinically relevant disease. These cloned cell lines may thus represent an intermediate stage in the progression to malignancy. The pathology report revealed no evidence of malignancy or PIN lesions and the patient has no further symptoms.

To summarise, characteristics of cell lines that are successfully immortalised have the ability to grow out with

the usual lifespan and can be cultured indefinitely. However, immortalised cells will not exhibit characteristics similar to transformed cells which include; anchorage-independent growth, high c-myc expression, extensive abnormal chromosome aberrations and the ability to form tumours in immunocompromised animals.

Our results suggest that the P21PZ and P21PZ (clone-a) cell lines are transformed cell lines and P21TZ being a normal cell line. P21TZ is a cell line immortalised by the overexpression of *cdk4* and *hTERT* and a normal cell model for human prostate tissue. P21PZ and P21PZ (clone-a) are cell lines immortalised by the overexpression of *cdk4* and *hTERT* but naturally transformed (not irradiated) tumourigenic cell model for human prostate tissue. The manipulation to create these cell lines has used two human genes and no irradiation to transform them therefore they are a very good model to study the effects of prostate cancer as they have no viral induced abnormalities and all cellular changes are occurring spontaneously in the transformed lines. In comparison, we have an immortalised radiation transformed cell line from the transition zone of the prostate indicating that prostate cells can be transformed by gamma radiation.

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