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Serially heterotransplanted human prostate tumours as an experimental model

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Abstract

Preclinical research on prostate cancer (PC) therapies uses several models to represent the human disease accurately. A common model uses patient prostate tumour biopsies to develop a cell line by serially passaging and subsequent implantation, in immunodeficient mice. An alternative model is direct implantation of patient prostate tumour biopsies into immunodeficient mice, followed by serial passage *in vivo*. The purpose of this review is to compile data from the more than 30 years of human PC serial heterotransplantation research. Serially heterotransplanted tumours are characterized by evaluating the histopathology of the resulting heterotransplants, including cellular differentiation, karyotype, marker expression, hormone sensitivity, cellular proliferation, metastatic potential and stromal and vascular components. These data are compared with the initial patient tumour specimen and, depending on available information, the patient's clinical outcome was compared with the heterotransplanted tumour. The heterotansplant model is a more accurate preclinical model than older generation serially passaged or genetic models to investigate current and newly developed androgen-deprivation agents, antitumour compounds, anti-angiogenic drugs and positron emission tomography radiotracers, as well as new therapeutic regimens for the treatment of PC.

Keywords: experimental model • heterotransplant • nude mice • prostate • xenotransplant

Introduction

Prostate cancer (PC) is the second most common type of cancer among men in the United States, and it is the second leading cause of cancer death in men. In 2009, an estimated 192,280 men will be diagnosed with, and 27,360 men will die of PC [1]. There are several therapeutic approaches to treat PC. Surgery (radical prostatectomy) and radiotherapy are therapies with 5-year survival rates greater than 90% [2, 3]. The most significant advance in PC therapy was the observation by Huggins and coworkers [4] that PC is a hormone-dependent disease. In this context, androgen deprivation therapy (ADT) can be achieved using several treatment options. Bilateral orchiectomy has response rates from 50% to 80% [5, 6]; however, there are severe and irreversible side effects like loss of libido, impotence, hot flashes, osteoporosis and muscle wasting. In addition, ADT although temporarily effective as

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an antitumour therapy, androgen independent disease develops. In this case, ADT becomes only palliative. It is in this context the need for research and the development of relevant models for PC.

ADT also may be achieved by treatment with oestrogens, like diethylstilbestrol (DES) or stilbestrol. Clinically, oestrogen therapy is based on the negative feedback loop of testosterone (T) regulation by inhibiting the release of luteinizing hormone-releasing hormone (LH-RH) in the hypothalamus. However, DES has severe side effects like cardiovascular and thromboembolic complications [7]. LH-RH agonists, such as leuprolide, goserelin and buserelin, are another group of drugs used for ADT. The sustained stimulation of the pituitary gonadotrope desensitized the LH-RH receptor (LH-RH-R), resulting in high levels of T in serum (flare phenomenon) initially, which soon fell to levels similar to those achieved by orchiectomy. Loss of libido, hot flashes and flare phenomenon are described side effects; pre-treatment with antiandrogens (AA) avoids the flare phenomenon. LH-RH antagonists inhibit the LH-RH-R, stopping the production of LH by pituitary gonodatrope cells and, subsequently, of T since, without LH stimulation, Leydig cells in the testis do not synthesize T. Cetrorelix, orgalutran and abarelix belong to this family of compounds.

AA are another group of drugs used in ADT. The non-steroidal agonists block T and 5α-dihydrotestosterone (DHT) from interacting with the cytosolic androgen receptor (AR), which is normally translocated into the nucleus, resulting in cell proliferation and inhibition of apoptosis. Also, in the pituitary gonadotrope may disassociate LH secretion from its negative feedback control, resulting in an elevation of LH and serum T levels. The serum T preserves libido and potency in patients. However, AA monotherapy (*e.g.* flutamide, bicatulamide and nilutamide), compared to orchiectomy, has an inferior response rate. The treatment of locoregionally advanced PC (stage T3) involves combination therapies. Radiation therapy combined with ADT, results in a significant improvement in patient progression-free survival [8]. However, radical prostatectomy combined with neoadjuvant ADT does not improve surgical outcome [9]. The management of persistent and recurrent disease after initial definitive therapy includes failure after radiation therapy, salvage surgery and ADT. Treatment options after radical prostatectomy failure include adjuvant radiation therapy in combination with ADT [10]. Approximately, less than 3% of PC will be metastatic at the time of diagnosis (stage T1–4NXM$^+$) [11].

However, persistent and recurrent disease results in PC progression to androgen-independent disease and metastases. Unfortunately, docetaxel is the only chemotherapeutic agent in clinical use for this later stage of PC, combined with other antitumour agents like estramustine or prednisone [12]. In this context, development of new PC models is necessary.

An interesting PC animal model is the transgenic adenocarcinoma of mouse prostate (TRAMP). However, TRAMP mice in addition to glandular tumours of the prostate, consistently develop phyllodes-like epethelial-stroma (ES) tumours, which may become fully malignant and metastasize [13]. In examining metastases from TRAMP mice, researchers must distinguish the tumour type to determine whether the tumour is metastatic from the seminal vesicle ES or from a glandular tumour of the prostate. In this case, the primary site may influence the interpretation of results [13].

There are increasing experimental data demonstrating that, in some cases, developing artificial *in vitro* cell lines from human cancers results in distinct and irreversible loss of important biological properties originally present in the tumour. In gene expression studies of small cell neuroendocrine carcinoma of the lung, some genes have been shown to undergo irreversible changes in expression after the cells are cultured *in vitro*. Furthermore, expression patterns for a significant number of genes were not restored when the derivative cell line was returned to growth *in vivo* as a xenograft [14]. More detailed examples have been described [15]. Also, cell lines are sometimes mis-identified. For example, there has been controversy recently about the true origin of a human breast cancer cell line [16]. These concerns have made a prestigious scientific journal adopt a new policy for reporting data on cell line origin and authentication [17]. To evaluate the PC heterotransplant as an experimental model, there are two important characteristics that it must possess. First, the resemblance between the serially heterotransplanted tumour in the host mouse resembles the initial patient specimen must be determined accurately. Second, the fidelity with which the heterotransplant model reproduces the clinical outcome observed in patients must be characterized. This defines the predictive value of the heterotransplant model which ultimately has to predict how human beings will respond to new developed therapies.

Serially heterotransplanted human tumours in immunosuppressed mice: similarity to the tumour of origin

Note: In the text, donor tissue was implanted in the subcutaneous space of the host mouse (considered the standard location) unless specifically stated otherwise.

Cytological and histological analysis

Several human PC tumours, of varied degrees of differentiation, have been serially heterotransplantated. All of them retain the original morphology and histological differentiation of the original tumour during all passages in the host mice. This has been demonstrated with moderately differentiated carcinomas [18–22], moderately to poorly differentiated carcinomas [23, 24], poorly differentiated carcinomas [22, 25–30] and poorly differentiated carcinomas from testicular metastasis [31], from bone metastasis [32, 33] and from lymph node metastasis [22, 30, 34, 35]. Remarkably, the histological similarity between the original tumour and the serially heterotransplanted tumour has been verified in some cases for more than 30 passages [22, 23, 29, 30, 33, 36–38]. Similar results have been reported for poorly differentiated carcinomas when

serially heterotransplanted into the subrenal capsule [39, 40]. These results have been reported using athymic nude mice as a heterotransplant host and severely immunologically deprived mice strains; intact male and female mice and orchiectomized mice hormonally supplemented with T- or DHT-pellets. Matrigel [41] in the second passage heterotransplant tumour is not necessary for the heterotransplant to take again [33]. Importantly, the organization of the original prostate tumour is preserved in the heterotransplant even when the tissue was cryopreserved before transplantation [42].

Karyotype

Several karyotypes have been determined for serially heterotransplanted tumours like PC-82 [43], LAPC-3 and LAPC-4 [30], PCa1 and PCa1-met [40], and CWR22 and CWR22R [44] heterotransplants. The human heterotransplants LuCaP 23.8, 23.12, 35, 41, 49, 58, 69, 70 and 73 have contained 13 chromosomal aberrations (5 gains and 8 losses) per case. The chromosome arms that most often contained losses were 2q, 5q, 6q, 13q and 18q, while gains occurred most frequently in 7q, 8q and Xq. These regions are often altered in advanced PC in patients [45].

Marker expression

Prostate-specific antigen (PSA) expression has been detected in the patient's original tumour and in the serially heterotransplanted tumour in mice in the apical cell cytoplasm adjacent to small glandular lumina [31]. Csapo and coworkers [46] have observed for PC-82, PC-EW and PC-EG heterotransplanted tumours that, the larger the tumour volume, the higher the serum PSA concentration of the tumour-bearing mice. Similar observations have been reported for LuCaP-23 heterotransplants [47], KUCaP heterotransplants [48] and BM-18 heterotransplants [33]. Similar results have been obtained for the prostatic acid phosphatase. As determined by tumour tissue staining, PSA has been found from passage to passage for the serially heterotransplanted tumours PC-82, PC-EW [49], LuCaP [47], CWR22 [50] and for several tumours obtained from distant metastases described by Rubin and coworkers [51]. Similarly, subrenal capsule-implanted tumours maintained strong PSA expression, at a level similar to the original specimen, even after being passaged three times [39]. The serially heterotransplanted tumours PC-295, PC-310, PC-329, PC-346 and PC-374, as well as the original specimen, stained positive for PSA from passages fifth to eighth. Similar results have been reported for the BM-18 heterotransplant from early to late passages [33]. On the contrary, the androgen-independent heterotransplanted tumours PC-324 and PC-339 lost PSA expression during heterotransplantation, since the original specimens stained positive for PSA. In this case, the loss of PSA expression in both heterotransplanted tumours between passages fifth to eighth may be caused by an *in vivo* selection of primarily PSA^{-} tumour cells [22].

Other PC markers

The adenosine deaminase complexing protein was expressed at the same level in the original tumour as in the serially passaged heterotransplanted tumours [52]. Similarly, the original tumour stained positive for keratin, epithelial membrane antigen and Leu-7 (CD57), as did the PAC-120 heterotransplant [34]. Finally, pan-cytokeratin and cytokeratin-18 were expressed at similar levels in the serially passaged heterotransplanted tumours as in the original tumour [27].

Tumour cell proliferation and frequency of mitosis

The serially heterotransplanted tumour HONDA has more mitotic cells than the original tumour [31]. On the other hand, a serially heterotransplanted PCa1 tumour maintained a similar proliferation rate as the original tumour as determined by Ki-67 staining [39]. Using the proportion of cells in different phases of the cell cycle to determine tumour cell proliferation reveals that the PC-82 heterotransplanted tumour has a similarly slow growth rate to tumours in patients. The fraction of cells in the G_0/G_1 phase was 85–90%, while the fraction of cells in the G_2/M phase and S phase were 8% and less than 5%, respectively [53]. Similarly, the percentage of Ki-67^{+} stained cells for the same heterotransplanted tumour are 16.1% and 10.4% as determined by Galle and coworkers [54] and van Weerden and coworkers [53], respectively. Interestingly, the result of the percentage of cells in proliferation determined by Ki-67 staining paralleled with the result of the percentage of cells in proliferation determined by bromo deoxy-uridine incorporastion (BdU). In addition, the growth of the heterotransplanted tumour correlated with the percentage of BdU^{+} cells [53]. The PC-EW heterotransplant has a percentage of proliferating cells determined by Ki-67 staining of 5.2%. When the percentage is determined by the BdU incorporation assay is 3% and 5.4% as determined by van der Weerden and coworkers [29] and van der Weerden and coworkers [53], respectively. Similar proliferation rates could be measured by other methods such as staining for PCNA with similar results to Ki-67 expression and BdU incorporation [55].

Vasculature

At the first passage in the host mice, the percentage of human vessels in the viable PC heterotransplants was $79.3 \pm 4.8\%$ [56]. However, long-term serially passaged PC heterotransplants contain mostly mouse vessels. This human-to-murine vessel substitution may occur due to a serial dilution of human angiogenic signalling molecules, and the cells that produce them, as the heterotransplants are serially harvested, fragmented and re-implanted. Consequently, the human vasculature present in the PC heterotransplant may be replaced over time if the tumours are serially heterotransplanted long term [56]. In these cases, early cryopreservation of heterotransplants preserves the majority of human

vasculature [42]. The PC-82 heterotransplant has been used as a tumour model to investigate the anti-angiogenic drug linomide [57]. The CWR22R heterotransplant has been used to evaluate the experimental anti-angiogenic humanized antibody anti-vascular endothelial growth factor (VEGF) bevacizumab alone and in combination with 5-fluorouracil [58], and the novel compound tasquinimod, an inoline-3-carboxamide with promising antitumour activity when combined with docetaxel [59].

Stromal compartment

After long-term heterotransplantation, PC-82 and PC-EW tumours present as small islands of keratin-negative and vimentin-positive cells in the tumour parenchyma. These cells are stromal, and account for less than 10% of the tumour tissue. Histologically, they appear to be murine in origin [21, 37]. Other authors have described up to 2% of proliferative cells in PC-82 heterotransplanted tumours are stromal, and of murine origin [29]. Similarly, immunohistochemical analysis using anti-human HLA-A, B and C antibodies revealed strong reactivity in 100% of the epithelial cells in the LuCaP-35 heterotransplant. However, the stromal tissue inside the tumour did not stain for any of these HLAs [38]. The human-to-murine stromal cell substitution in the heterotransplant may occur in a manner similar to the human-to-murine vascular substitution previously described. As in that case, early cryopreservation of the heterotransplants may help to preserve the majority of human stroma cells.

Heterotransplant hormone dependency

Androgen dependent

Pioneering work from Williams and coworkers [26] demonstrated that, when tritiated T was administered to PC heterotransplant bearing-mice, the xenografts maintained their ability to take up and concentrate it two to three times greater than the control tissue. Many researchers have observed that heterotransplants grow on males or on orchiectomized males supplemented with T, but not on females [18, 21–24, 29, 34, 35, 50, 60], an important part of androgen dependence. However, when female mice were supplemented with T-pellets, the heterotransplanted tumour grew in a continuous pattern [31]. Another piece of therapeutically relevant evidence showed that orchiectomy of heterotransplanted tumour-bearing mice results in shrinking of the growing tumour. This androgen-dependent tumour shrinkage has been described for PC-82 [21], PC-EW [23], HONDA [31], TEN12 [24], PC-EG [46], CWR22 [50], LuCaP-35 [38], KUCaP [48] and BM-18 [33] heterotransplants. Interestingly, treating the HONDA heterotransplant donor patient with an ADT-like oestrogen therapy caused a partial clinical response that prolonged the patient's lifespan for more than 1 year [31]. As previously stated, oestrogens are effective in treating PC. Further evidence shows that regular administration of oestrogens, like 17-β-estradiol, to intact mice at the time of tumour heterotransplantation, or when the tumour is in an exponential growth rate, results in tumour shrinkage. This antitumour effect has been described for the PC-82 [21, 61], PC-EW [23] and PC-EG [46] heterotransplant. Interestingly, treating the donor patient of the PC-EW heterotransplant with a combination of hormonal and radiation therapy resulted in a remission of the tumour [23]. The behaviour of the heterotransplanted tumours matched the patient's clinical outcome.

As expected, PC-82 and PC-EW heterotransplanted tumours express AR mRNA and protein [62]. When the PC-82 heterotransplanted host mice receive a T-pellet supplement, the tumour growth rate and AR nuclear concentration increased [63]. Other androgen dependent and serially heterotransplanted tumours (PC-295, PC-310, PC-329, PC-346 and the variants PC-346P and PC-346B) all stained positive for AR, like the original specimens [22, 60]. The same was true for the heterotransplanted tumours described by Presnell and coworkers [42], and the KUCaP [48], BM-18 [33] and PCa1 [39] heterotransplants. The heterotransplanted PC-82, PC-EW, PC-295, PC-310 and PC-329 tumours express the AR in more than 80% of their tumour cells. However, after androgen withdrawal, these heterotransplants had reduced AR expression, in less than 30% of their cells [64]. The PC-82 heterotransplant had decreased nuclear hAR expression 5 days after androgen withdrawal. However, after T supplementation, nuclear hAR expression was restored rapidly. Surprisingly, the hAR mRNA levels do not substantially change during the 5 days of androgen deprivation, as demonstrated by S1-nuclease protection assay. Therefore, the decrease in the receptor expression is caused either by autoregulation of hAR expression and increased translation, or by a stabilization of the receptor protein [65].

Interestingly, the AR expressed by the KUCaP heterotransplant, obtained from a liver metastases, has a mutation (W741C) like the original metastatic lesion [48]. The administration of the AA bicatulamide to orchiectomized tumour-bearing mice resulted in tumour growth and an increase in serum PSA concentration, paralleling tumour volume increases, showing that the AA promotes the growth of the W741C mutant heterotransplanted tumour. Identical results were previously found with the patient, who was treated with chemoendocrine therapy then ADT (LH-RH combined with bicatulamide) just before death [48]. It is important to note that AR expression persists in clinical PC despite progression to androgen-independent state. AR mutations are not common in primary PC that has not been treated with an ADT. However, during treatment, AR undergoes genetic alterations, including AR gene amplification. In addition, the frequency of point mutations in the AR gene is significantly increased (from 10% to 30%) in tumours after maximal ADT. As informative, another androgen-dependent heterotransplant, CWR22, obtained from a primary PC tumour, has a point mutation at H874Y [28].

Androgen-dependent heterotransplanted tumours develop important changes after androgen withdrawal. At the histological level, in the PC-82 heterotransplant, epithelial height decreases with focal desquamation, the acinar lumina widens (dilated glandular lumen), and the tumour shrinks and foci are cleared. At the cytological level, the cytoplasm is vacuolized and apparent partly vacuolized and pyknotic nuclei with less distinct nucleoli,

compared to control heterotransplanted tumours in intact mice [21, 23, 66–68]. Moreover, the PC-EW heterotransplant tumour responded more substantially to androgen deprivation. Severe destruction of the glandular structure of the tumour parenchyma and massive necrosis of epithelial tumour tissue affecting 70–80% of the tissue was observed [29]. These changes are similar to those observed in PC tumours from surgically or medically androgen-deprived patients.

The orchiectomy of the host tumour-bearing mice causes a decrease in cell proliferation in the serially passaged PC-82 heterotransplant, even after 12 days of the androgen removal [29, 53]. Similarly, a study of the cell cycle phase showed no detected cells in S phase or G_2/M phase [53]. Concomitantly, the resultant tumour shrinkage was associated with an increase in the percentage of cells undergoing apoptosis [53]. However, other tumour cells maintained viability and could be stimulated to regrow after T re-supplementation. Similarly, androgen withdrawal of PC-EW tumour-bearing mice resulted in a severe tumour shrinkage, along with a decrease in cell proliferation and an increase in apoptosis and, more importantly, necrosis [29, 53]. Consequently, the PC-EW tumour regressed completely and could not be stimulated to grow after T re-supplementation [29, 64]. Similarly, apoptosis increased, as well as caspase-3 expression, after orchiectomy of the CWR22 tumour-bearing mice [69]. Remarkably, the PC-82, PC-EW and BM-18 heterotransplants, even under prolonged androgen deprivation, did not develop an androgen-independent variant [37, 33].

Androgen-dependent heterotransplants have been used as preclinical models to investigate the antitumour activity of several newly developed drugs. The PC-82 heterotransplant was used to evaluate some 5α-reductase agonists, like the 17β-*N,N*-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one (4-MA) [70] the Smith-Kline & French compound 105657 [71], the Δ^4-3-one-pregnane derivatives and the Δ^5-3β-ol-androstane derivatives [72], the LH-RH analogue coupled to doxorubicin, 2-pyrrolinodoxorubicin-[D-Lys_6]LH-RH [73] as well as two LH-RH antagonist, SB-75 [74] and [N-Ac-D-p-Cl-$Phe_{1,2}$,D-Trp_3,D-Arg_6,D-Ala_{10}]-LHRH [75], two anti- oestrogenic phenylkindoles [76], and an AR steroideal antagonist [77]. The LuCaP-35 heterotransplant has been used as a model to test the antitumour activity of the LH-RH analogue AN-207 alone [78] and in combination with growth hormone-releasing hormone antagonists [79] and the bombesin analogue AN-215 [80]. This heterotransplant also has served as a model to investigate the insulin-like growth factor receptor monoclonal antibody A12, alone and in combination with surgical orchiectomy [81, 82]. In addition, this model has been used to evaluate a novel strategy based on the inhibition of the enzyme 17α-hydroxylase/17,20-lyase (CYP17) that catalyses the production of T in testes and adrenal glands. The androstene derivative 17-(5′-isoxazolyl) androsta-4,16-dien-3-one, a non-competitive inhibitor of CYP17, is a potent inhibitor of androgen synthesis, and is effective in reducing the growth of the heterotransplant [83]. The heterotransplants LuCaP-35, -49 and -73 have been used to test the antitumour activity of 17-β-estradiol [84]. The anti-mitotic agent vindesine has been investigated for its antitumour activity, using three human PC tumours heterotransplanted into mice [80]. Finally, another androgen-dependent heterotransplant, LACP-9, has been used to evaluate the antitumour and antimetastatic efficacy of the monoclonal antibody directed against prostate stem cell antigen [86].

Partially androgen dependent

Heterotransplanted tumours LuCaP 23.1 and LuCaP 23.12 responded heterogeneously to host mice orchiectomy. One group of orchiectomized mice responded with no increase in tumour volume or shrinking of the tumour. On the other hand, in two other groups, tumour growth finally resumed after a delay of several days [47]. Androgen withdrawal increased the mouse's lifespan several fold, as compared to control intact tumour-bearing mice. In the latter groups, the AR was found in the tumour cell nuclei, in a heterogeneous staining pattern in which the mRNA for 5α-reductase isotype 1, was found in prostate epithelial cells. Unfortunately, the donor patient of both heterotransplants was diagnosed and died from hormone refractory disease [47]. The heterotransplanted tumour's behaviour matched the patient's clinical outcome. Similar tumour heterogeneous response has been reported with LuCaP-35 [38], LAPC-4 [30], PAC-120 [34], TEN12-C1 and -C2 [87], PAC-120 [88], and CWR22 [89] heterotransplants. Unfortunately, the donor patient of the PCA-120 heterotransplant received chemotherapy, with adjuvant AA therapy, but progressed to hormone-refractory disease [34]. Similarly, the CWR22 heterotransplant responded heterogeneously resulting in the androgen-independent tumour heterotransplant CWR22R [89]. In all these heterotransplants, the relapsed androgen-independent tumour growth could be anticipated by an increase in PSA serum concentration.

The LAPC-4 heterotransplant contains wild-type sequences in exons 2 to 8 of the AR. These sequences remain wild-type in the androgen-independent LAPC-4 variant, providing evidence that the progression to an androgen-independent PC can occur in the absence of AR mutations. Interestingly, most patients who donated specimens for the LAPC heterotransplant series had undergone some form of ADT (surgical or medical) and progressed to hormone-refractory disease at the time the tumour specimens were obtained [30]. The LuCaP 23, PAC-120 and LAPC-4 heterotransplanted tumours behaviour matched the patient's clinical outcome. All in all, these results demonstrate that androgen-independent tumour variants can be developed using heterotransplants, confirming the clinical transition from androgen dependent to an androgen-independent disease. The LuCAP 23.1 heterotransplant was used to evaluate the therapeutic efficacy of the anti-insulin growth factor 1 receptor monoclonal antibody, IMC-A12 [90], and of the potent differentiating agent phenylbutyrate [91].

The CWR22 heterotransplant was used to investigate the therapeutic efficacy of two histone deacetylase inhibitors, suberoylanilide hydroxamic acid [92] and pyroxamine [93]. The same heterotransplant model was used to test the monoclonal antibody MLN2704 against the prostate specific membrane antigen

(PSMA), maytasinoid 1 [94]. In addition, it was used to evaluate the antitumour efficacy of the tamoxifen–quercetin combination [95], and the tamoxifen–trastuzumab (an anti-Her2/ν monoclonal antibody) combination [96]. Bristol-Myers-Squibb has used this heterotransplant to investigate the orally active taxane BMS-275183 [97]. Similarly, the farnesyltransferase inhibitor lonafarnib increases the antitumour activity of docetaxel in combination [98], and of the oestrogen receptor B inhibitor raloxifene, currently used as an adjuvant in breast cancer [99]. The antitumour efficacy of the selective inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase domain, initially named ZD1839 and later gefitinib alone [100], and in combination with bicalutamide [101], has been tested using the CWR22 heterotransplant. Recently, a novel series of (2.2.1)-oxabicyclo imide-based AR antagonists have been tested using this heterotransplant [102], as well as the monoclonal antibody directed against human TMEFF2, a protein highly expressed in PC, alone or conjugated to auristatin E, a known tubulin inhibitor [103].

The LAPC-4 heterotransplant has been used to investigate the C-17-heteroaryl steroidal CYP17 enzyme inhibitor [104] and the newer inhibitor 3β-hydroxy-17-(1H-benzimidazole-1-yl) androsta-5,16-diene, which inhibits not only the enzyme but also the AR, resulting in potent antitumour activity compared to bicatulamide [105]. Also, this heterotransplant has been used to develop more specific 17-β-estradiol that result in fewer side effects in patients. In this context, the antitumour efficacy of the estradiol analogue, 17α-estradiol [106] as well as of selenite [107], was investigated. Finally, using this heterotransplant, a series of bifunctional compounds have been synthesized with LH-RH agonists linked to one side and a synthetic ligand to the co-activator binding site of AF-2 domain to the other, resulting in potent LH-RH antagonist activity [108]. Similarly, the PAC-120 heterotransplant, and its androgen-independent variants, were used to evaluate the therapeutic efficacy of mitoxantrone, estramustine phosphate and docetaxel [109], docetaxel combined with trastuzumab and a Grb_2-SH_3 ligand named peptidimer-c [110].

Positron emission tomography (PET), a nuclear medicine imaging technique, is used to diagnose, detect and stage primary tumours and metastases, as well as assess the effectiveness of a treatment and monitor disease progression after prostatectomy, radiotherapy or ADT, especially in locoregionally advanced (stage T3) and persistent recurrent disease. In clinical oncology, glucose analogues are widely used as radiotracers because they specifically accumulate in metabolically active tumours. The CWR22 heterotransplant has been used as a preclinical experimental model to investigate several radiotracers. Changes in tumour metabolism were assessed by (^{3}H)-deoxyglucose accumulation in a tumour using PET scanning as an early indicator of treatment efficacy [111]. Other radiotracers been used for the PET assessment of androgen modulation of tumour glucose metabolism, acetate uptake and prostate-specific membrane antigens are 2-(^{18}F)-fluoro-2-deoxy-D-glucose (FDG), ^{11}C-acetate [112] and ^{64}Cu-PSMA [113]. ^{68}Cu-PSMA monoclonal antibody specifically targets and traces PSMA expressing prostate tumours ^{68}Cu-PSMA monoclonal antibody specifically targets PSMA expressing prostate tumours [113]. FDG is useful in imaging to evaluate the response to ADT, and in the early prediction of hormone refractoriness in patients with metastatic PC [114, 115]. However, ^{11}C-acetate has high sensitivity for detecting primary and metastatic PC that are poorly detected with FDG. To address this deficiency ^{18}F-fluoroacetate, an acetate analogue with a longer radioactive half-life which may be a useful alternative to ^{11}C-acetate for the detection of PC, was developed [116]. 3′-deoxy-3′-^{18}F-fluorothymidine is another radiotracer used to detect and monitor the therapeutic effect of ADT in PC [117]. Finally, nuclear magnetic resonance spectroscopy using the CWR22 heterotransplant provides a method to monitor metabolic changes of tumour response to radiation therapy [118].

Androgen independent

In some serially implanted heterotransplants, tumour growth is not affected by the sex of the host mouse or androgen supplementation showing an androgen-independent growth [22, 32]. Interestingly, in the report from van Weerden and coworkers [22] the patient's tumour was diagnosed as hormone-refractory PC and in the report from Graham and coworkers [32] the original patient's tumour became hormonally unresponsive. In both cases, the heterotransplanted tumours behaviour matched the patient's clinical outcome. Several androgen-independent heterotransplants have been developed from initial androgen-dependent parental heterotransplants, such as TEN12-F [119]. TEN12 cells were implanted into female mice, and passaged and maintained in female mice, producing the TEN12-F heterotransplant. Re-introduction of cells from the TEN12-F heterotransplant and their subsequent passage into male mice resulted in the androgen-independent heterotransplant TEN12-FM [119].

LAPC-3 is an androgen-independent serially heterotransplantated tumour that grows regardless of the hormonal background of the host mouse. The tumour contains wild-type sequences in exons 2 to 8 of the AR gene providing further evidence that androgen-independent PC progression can occur in the absence of AR mutations. As previously stated, most patients who donated their tumour for the LAPC series of tumours had undergone some form of ADT and showed tumour progression to a hormone-refractory disease at the time of the tumour specimen collection [30].

Another androgen-independent heterotransplanted tumour is PC-135 [29, 62]. In PC-135 orchiectomized tumour-bearing mice, the plasma T and 5-DHT concentrations were nearly undetectable, but the tumour grew [37]. This heterotransplant does not express either AR mRNA or the corresponding protein, similar to the androgen-independent PC-133 serially heterotransplanted tumour [62]. Three more serially heterotransplanted tumours capable of growing in female host mice are PC-324, PC-339 and PC-374. However, the first two (PC-324 and PC-339) have lost AR expression during heterotransplantation, since the original specimens stained positive for it. In this case, the loss of the AR expression may be due to *in vivo* selection of primarily AR^{-} tumour cells [22]. In contrast, the heterotransplanted tumour PC-374, obtained from a scrotal skin metastasis, stained positive for AR in the original

specimen and in the serially heterotransplanted tumours (passages fifth to eighth). The donor patient was treated with LH-RH agonists, radiation therapy and AA before specimen collection [22, 64].

From the parental PC-346P and PC-346B heterotransplants obtained by van Weerden and coworkers [22], Marques and coworkers [120] have derived three more heterotransplants named PC-346I, PC-346SIcas and PC-346BI all serially passaged in female mice. The PC346 heterotransplant has a point mutation in the AR at T877A.

Androgen-independent heterotransplants are useful preclinical models to investigate new ADT agents. A novel rational design of AA for neutralizing AR function in androgen-independent PC has been proposed. Sign and coworkers [108] have developed a bifunctional approach to design LH-RH agonists linked to a synthetic ligand that recruits FK506-binding chaperone proteins (FKBPs) to the co-activator binding site of AF-2 domains, thereby sterically preventing binding of any co-activator proteins to the AR. As a result, the AR is locked in an antagonistic conformation.

In this context, the CWR22R heterotransplant has been used to investigate the antitumour activity of the microtubule-depolymerizing agent PC-SPES [121]. Similarly, the EGFR tyrosine-kinase inhibitor gefitinib alone [100] and in combination with bicalutamide [101] have been evaluated using this model, as well as the matriptase inhibitor CVS-3983 [122], the fungal metabolite FTY720s [123], the garlic-derived compound S-allyl cysteine [124], several herbal supplements [125], and a recombinant humanized monoclonal antibody directed against VEGF (rHu α-VEGF) [126].

Metastases

The LACP-4 tumour serially heterotransplanted into host mice was derived from a metastatic lymph node [27]. The authors found PSA mRNA in the lung of LACP-4 heterotransplant-bearing mice, and detected PSA mRNA^{+} cells in the peripheral blood, bone marrow and spleen in half of them. Another human prostate heterotransplanted tumour that developed spontaneous metastasis is the serially passaged PCa1 tumour, originally grown in the subrenal capsule [39]. The orthotopic heterotransplantation of the metastatic PCa1-met tumour, derived from the parental PCa1, developed metastasis in lymph nodes, lung, liver, kidney, spleen and in bone (in 43% of mice). Lung, liver, kidney and spleen metastasis stained positive for human AR and human mitochondria [39]. To increase the metastatic potential from heterotransplanted tumours, Corey and coworkers [38] implanted LuCaP-23.8 and LuCaP-35 tumour fragments into the mouse's coagulating gland, which parallels the seminal vesicle. By removing the orthotopic primary tumour when it was large, they were able to generate metastasis. Lymph node metastases were macroscopic, and tumour foci growing in distant tissues stained positive for PSA (LuCaP-23.8) or AR (LuCaP-35). Lung metastases were detected in 71% and 90% of mice transplanted with LuCaP-23.8 and LuCaP-35, respectively, as well as liver, diaphragm and pancreatic metastases. LuCaP-23.8 induced metastases producing low levels of serum PSA and osteolytic and mixed osteoblastic lesions, while LuCaP-35 metastases produced high serum PSA levels and osteoblastic lesions. All metastases stained positive for proteins affecting bone cells, like osteoprotegerin, RANK (receptor activator of nuclear factor κB) ligand, and parathyroid hormone-related protein [35]. The LuCaP-23.1 heterotransplant also has been used as a model for metastases to test an osteoprotegerin inhibitor on intra-tibial metastatic growth [127]. As previously described, bone osteoporosis is a serious side effect of ADT in patients. The bisphosphonate compound zelodronic acid, an osteolysis inhibitor that increases bone mineral density, has been tested alone [38] or in combination with docetaxel [128]. Patients with PC metastases have fewer fractures and other bone complications when they take zoledronic acid than when they take placebo [129].

Conclusions

PC tumours serially heterotransplanted into mice have important properties present in the original patient tumour *in situ* like morphology, pathology, differentiation, secretory activity and tumour marker expression. Importantly, human tumour architecture is preserved, allowing the stromal-epithelial cell crosstalk. On the other hand, the human stroma and vasculature are substituted as serial passaging progresses. Early cryopreservation of the heterotransplanted tumours preserves the majority of human tumour stroma and vasculature. Serial heterotransplantation results in androgen-dependent and androgen-independent heterotransplants, and accurately reproduces the clinical transition from androgen-dependent to androgen-independent disease. In several cases, when patient information was available, the heterotransplanted tumour reproduced in mice the outcome observed in the patient, which demonstrates the predictive value of the heterotransplant model. In this context, the heterotransplantants have been used to evaluate new androgen deprivation agents, antitumour compounds, anti-angiogenic drugs and PET radiotracers, as well as new therapeutic regimens for the treatment of PC. Establishing a PC heterotransplant model represents a significant advance in the tools available to study PC.

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