Genetically modified macrophages expressing hypoxia regulated cytochrome P450 and P450 reductase for the treatment of cancer

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Abstract. This study describes a combined gene and cell therapy based on the genetic modification of primary human macrophages, as a treatment for cancer. Here, we have utilised the tumour-infiltrating properties of macrophages as vehicles to deliver a gene encoding a prodrug-activating enzyme such as human cytochrome P450 2B6 (*CYP2B6*) inside tumours followed by killing the tumour cells with the prodrug cyclophosphamide (CPA). Macrophages were transduced with an adenoviral vector that expresses human cytochrome *CYP2B6* via a synthetic hypoxia responsive promoter (OBHRE) and with human P450 reductase (P450R), via the CMV promoter. In the presence of CPA, these genetically modified macrophages showed increased cytotoxicity against various tumour cell lines compared to untransduced macrophages or macrophages transduced with *CYP2B6* alone. In human ovarian carcinoma xenograft models, the median survival of mice treated with genetically modified macophages plus CPA increased up to two-fold compared to the survival of mice treated with untransduced macrophages and CPA. Genetically modified autologous macrophages may be a feasible therapeutic option for the treatment of some solid tumours, such as ovarian cancer.

Introduction

Infiltration of macrophages into the hypoxic region of tumours is well recognized (1-5). Macrophages have been shown to infiltrate ovarian tumours, producing monocyte chemotactic protein-1 (MCP-1). Furthermore, these cells are present in significant numbers near the necrotic region where hypoxia down-regulates MCP-1 (6-8). Tumour associated macrophages (TAM) are mainly M2 macrophages that are 'corrupted' by cytokines, e.g. IL-10 produced by the tumour microenvironment and involved in type II immune responses (1,3,5,9). These macrophages typically express IL-10^{high}/ IL-12^{low}, CD163^{+}, scavenger receptor A (SR-A)$^{+}$ and mannose receptor (MRC1)$^{+}$ phenotypes (5,9). TAMs are also attracted to the tumour site by chemokines produced by the tumour cells where they promote angiogenesis leading to tumour growth and spread (5). Classically, interferon (IFN)-γ- and/or lipopolysaccharide (LPS)-activated M1 macrophages which are involved in type I reactions produce reactive oxygen and nitrogen intermediates that have been found to be cytotoxic to tumour cells *in vitro* (5,9,10). However, there is a lack of *in vivo* evidence to confirm this observation. Nevertheless, this *in vitro* observation suggests that the tumour infiltrating abilities of macrophages may be complimentary to a gene directed enzyme prodrug therapy (GDEPT) strategy for cancer treatment.

Our GDEPT strategy involves the prodrug-activating enzyme, cytochrome P450 (P450 2B6), which metabolises the prodrug cyclophosphamide (CPA) into the toxic metabolites, phosphoramide mustard and acrolein. These metabolites cross-link DNA and proteins leading to cell death. The 4-hydroxy intermediate metabolites are freely diffusible among tumour cells, thus enabling a relatively small number of P450-expressing cells to kill more of the surrounding non-P450 expressing cells via a mechanism known as the bystander effect. Direct delivery of the cytochrome *P450* gene into tumour cells followed by CPA treatment significantly inhibits tumour growth and this local activation of CPA in the tumour cells requires a lower therapeutic dose of the prodrug, thereby reducing the systemic toxicity caused by CPA conversion in the liver (11,12).

P450 reductase (P450R) catalyzes the electron transfer required for the P450 enzyme activity and this flavoenzyme is ubiquitously expressed in all cells including malignant cells (13). However, the wide variation in the endogenous expression levels of this enzyme means that it may be rate-

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limiting for CPA prodrug activation by P450 in certain cell types. This is confirmed in studies where overexpression of P450R substantially increases the potency of P450/CPA mediated tumour cell killing (11,12,14,15). Thus, overexpression of P450R can significantly increase the potency of a P450/CPA based GDEPT strategy. Here, we present data to show that the combination of GDEPT with tumour infiltrating macrophages may be useful for the treatment of cancers, such as ovarian cancer.

Materials and methods

Cells and culture media. Unless otherwise stated, all cell lines were purchased from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and were cultured at 37˚C, 5% CO_2 in DMEM (Sigma, Poole, UK) supplemented with 10% (v/v) fetal calf serum (FCS), 2 mM L-glutamine and 1X non-essential amino acids. The LS174T cells were cultured in EMEM (Sigma) instead of DMEM.

Generation of recombinant adenoviral vectors. The Ad-CMV-GFP (Ad-G) and Ad-CMV-CYP2B6 IRES GFP (Ad-P) adenoviral vectors were generated as previously described (16). The Ad-OBHRE-CYP2B6-CMVP450R (Ad-PR) vector was based on the Ad-OBHRE CYP2B6 vector (16). The *P450R* gene was subcloned from the pBlueScriptIISK-P450R plasmid (a gift from Mark Saunders, Manchester). The Ad-PR vector was generated as follows: two oligonucleotides (5'-GA TCCCGTAGGATATCGCTGACGA; GGCATCCTATA GCGACTGCTCTAG-5') were constructed with 5' *Bam*HI and 3' *Bgl*II ends containing an *Eco*RV site. These were cloned into the *Bgl*II site of pE1 CMV CYP2B6 1 to generate pKT74. The *Eco*RI-*Xho*I fragment from pBlueScriptIISK-P450R was excised and cloned into the pcDNA3.1Zeo$^+$ (Invitrogen) to generate pZeoP450R. Sequencing of this plasmid revealed four nucleotide changes in the P450R coding sequence compared to GenBank accession no. S90469 resulting in two amino acid changes. Next, the CMVP450R cassette was excised from the pZeoP450R plasmid as a *Bgl*II-*Psi*I fragment and cloned into the *Bgl*II-*Eco*RV site of pKT74 to create pKT73. The CMV promoter was then replaced with the HRE promoter as a *Sgr*AI-*Not*I HRE fragment from pE1 OBHRE CYP2B6 1 to generate pKT78. The HRE-*lac*Z-SV40 poly(A) region was excised from OBM40 as a *Kpn*I-*Psh*AI fragment and cloned into the *Kpn*I-*Hpa*I sites of AdMIRE (Invitrogen) to generate AdMIRE HRE *lac*Z which contains a *Pac*I site 5' of the left ITR. This plasmid was cut with *Sfi*I-*Psh*AI to excise the backbone region containing the *Pac*I site and cloned into pKT78 to generate the Ad-PR transfer vector. Recombinant adenoviral vectors were generated and purified as previously described (17).

BrdU cell proliferation assay. Cells were seeded at $1x10^5$ cells/well in a 12-well plate and transduced the following day with adenoviral vectors for 6 h. Cells were re-seeded into 96-well plates (Viewplate 96, Packard) at $2x10^3$ cells/well and appropriate amount of CPA was added the next day. Following incubation for 6-7 days the BrdU incorporation was measured using the BrdU assay kit (Roche) according to the manufacturer's instructions.

Clonogenic survival assay. The clonogenic survival of MDA231 cells co-cultured with macrophages transduced with Ad-CMV P4502B6 and Ad-CMV P450R vectors (MOI=1000) in the presence of CPA was determined as described previously (18). For the co-culture assays untransduced or transduced macrophages were seeded at a density of $5x10^2$ macrophages/well in a 48-well plate pre-seeded with T47D at a density of $5x10^3$ cells/well, giving a ratio of 1:10 macrophage/T47D. The cells were co-cultured overnight, after which CPA was added at a final concentration of 1 mM. After 10-14 days the clonogenic survival of T47D cells was determined as previously described (18).

Isolation of primary human monocytes and generation of macrophages. Primary human monocytes isolated from buffy coat (National Blood Service, Bristol, UK) were allowed to differentiate into macrophages and were transduced with the adenoviral vectors as previously described (16).

Detection of P450 2B6 and P450R proteins expressed by Ad-PR vector transduced cells. MDA231 cells were transduced with the Ad-PR vector and exposed to either normoxic (21% O_2) or hypoxic (0.1% O_2) conditions. Hypoxia treatment was carried out in a 0.1% O_2, 5% CO_2 humidified incubator (Heto, Camberley, UK) for 16 h at 37˚C. The detection of P450 2B6 protein was carried out as described previously (18). The same method was used for the detection of the P450R protein except that the primary and secondary antibodies used were the rabbit anti-P450R (1:2000 dilution) (Millipore, UK) and the HRP-conjugated goat anti-rabbit IgG (1:2000 dilution) (DAKO, Ely, UK), respectively.

Measurement of the effect of CPA on T47D spheroids infiltrated with genetically modified primary human macrophages. T47D tumour spheroids were prepared as previously described (16) and were incubated with $2x10^4$ macrophages transduced with the different adenoviral vectors (MOI 500) for 2 days. Infiltrated spheroids were incubated in the presence of 1 mM CPA and exposed to hypoxia (0.1% O_2, 37˚C, 5% CO_2) for 65 h. The medium was then carefully aspirated and fresh medium ± 1 mM CPA was added and the spheroids were incubated for 14 more days. Photographs of the whole spheroids exposed to the different treatment regimens were taken with a light microscope. Spheroid diameters were determined by averaging the measurements from five T47D spheroids in each condition.

Evaluation of the effect of genetically engineered macrophages on the survival of mice implanted with HU or TOV21G tumour xenograft. For the HU tumour xenograft model, 60 female nude mice (6-8 weeks old) were injected intraperitoneally (i.p.) with 0.2 ml HU-ascites in 2% FCS supplemented RPMI-1640 media on day 0. On day 7, mice received TNFα (200 ng/mouse, 0.1 ml) i.p. daily for 7 days to allow HU to convert from the ascitic form into a solid tumour. On day 15, mice were randomised into 6 groups of 10 mice and each mouse was injected with either 0.2 ml of formulation buffer (saline), $2x10^6$ untransduced macrophages, or $2x10^6$ Ad-PR transduced macrophages. Each mouse was injected i.p. with either 0.2 ml of PBS or CPA (5 mg/ml) on

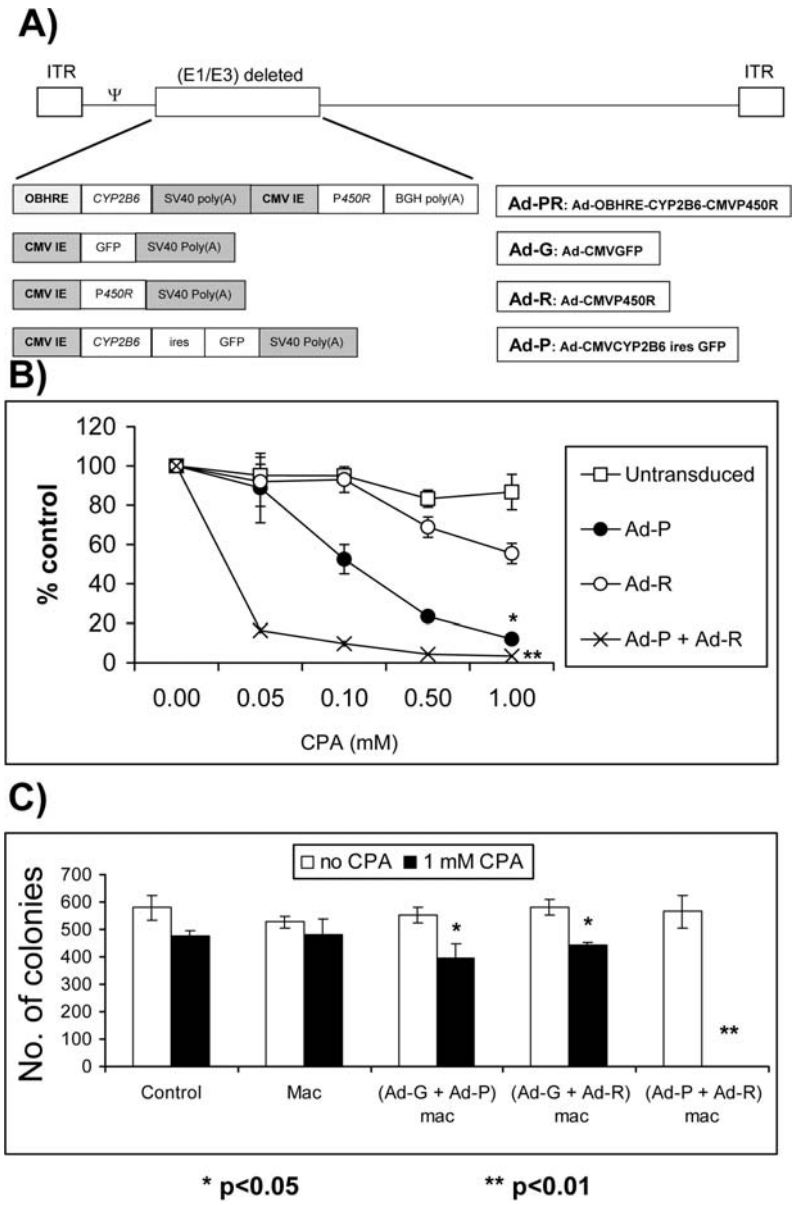


Figure 1. (A) The schematic diagram shows the configuration of the first generation recombinant adenoviral vectors used in this study (CYP2B6, human P450 2B6; P450R, P450 reductase; CMV IE, CMV immediate early promoter). (B) The potency of Ad-P450/CPA on the MDA231 human breast carcinoma cell line was enhanced by the co-transduction of the Ad-P450R vector (MOI=1000). The graphs show the mean % control (BrdU incorporation) ± standard deviation (SD) of a representative of 3 individual experiments. (C) The clonogenic survival of T47D cells is inhibited by the co-culture of macrophages transduced with Ad-CMV P4502B6 and Ad-CMV P450R vectors (MOI=1000) in the presence of CPA. Mac, untransduced macrophages; (Ad-G + Ad-P) mac, (Ad-G + Ad-R) mac and (Ad-P + Ad-R) mac, macrophages transduced with Ad-G + Ad-P, Ad-G + Ad-R and Ad-P + Ad-R vectors respectively. The number of surviving colonies with 1mM CPA treated (Ad-P+Ad-R) mac group is 0.33±0.67 (n=3). The graph shows the mean % control (clonogenic survival) ± SD of a representative of 3 experiments.

days 17 and 19 and weekly thereafter. The results were analysed by Kaplan-Meier survival analysis.

For the TOV21G tumour xenograft model, 80 female nude mice (6-8 weeks old) were injected with $2.5x10^6$ TOV21G cells i.p in 0.2 ml PBS on day 0. Mice were divided on day 44 into 8 groups of 10 mice and each mouse was injected with either 0.2 ml of formulation buffer, $2x10^6$ untransduced macrophages, $2x10^6$ Ad-PR transduced macrophages or Ad-PR vector [10^9 infectious units (IUs) per mouse]. Then each mouse was injected i.p. with either 0.2 ml of PBS or CPA per (5 mg/ml) on days 46 and 49 and weekly thereafter. The results were analysed using the Kaplan-Meier survival analysis.

Results

Ad-CMV P450R enhances the potency of the Ad-CMVP450 CYP2B6/CPA GDEPT strategy in vitro. In this study a series of recombinant first generation adenoviral vectors were constructed, shown diagrammatically in Fig. 1A. To confirm that overexpression of P450R enhances the anti-tumour effect of P450/CPA, an adenoviral vector expressing P450 (Ad-CMV-CYP2B6 IRES GFP, Ad-P) was evaluated in combination with an adenoviral vector expressing P450R (Ad-CMV-P450R, Ad-R) in a BrdU incorporation assay in the MDA231 breast carcinoma cell line (Fig. 1B). The combination of Ad-P and Ad-R was significantly more potent

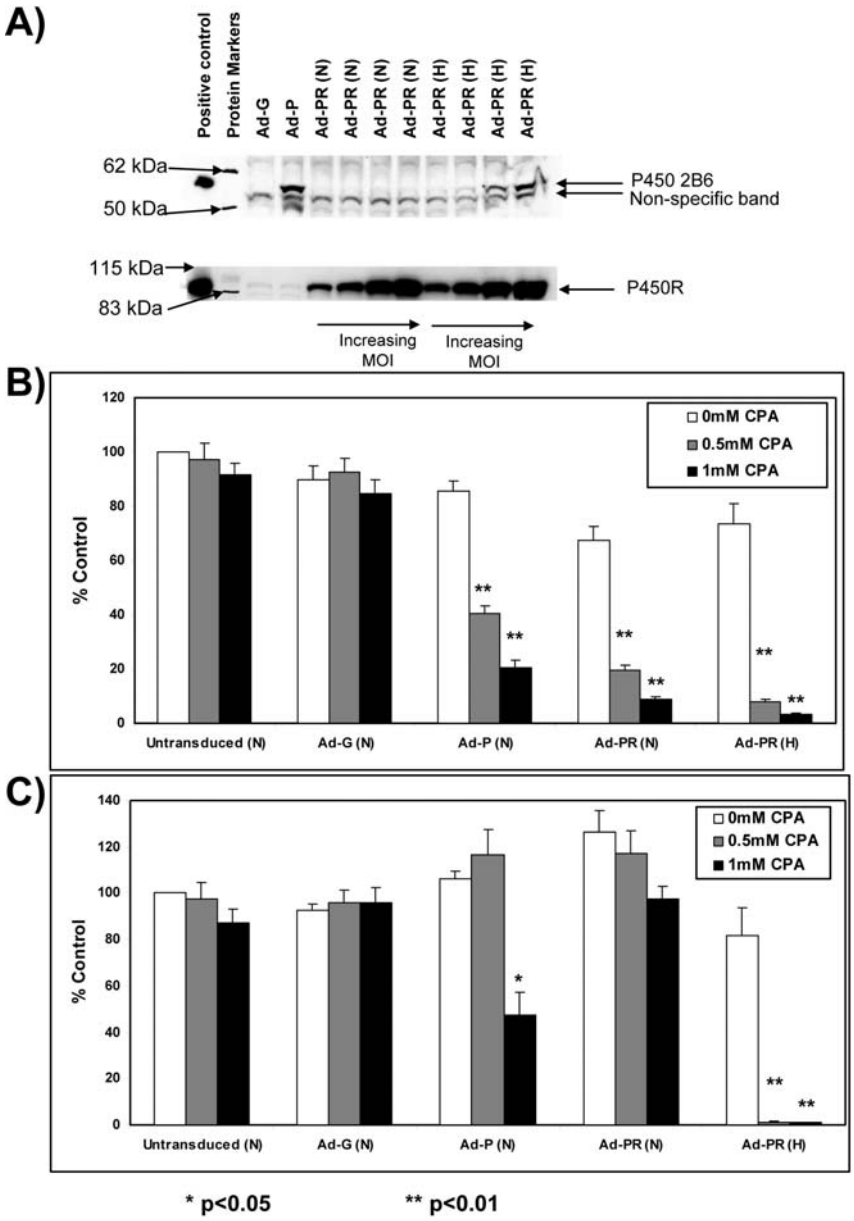


Figure 2. Effect of a recombinant adenovirus expressing both P450 and P450R on the proliferation of tumour cell lines. (A) Expression of P450 2B6 and P450R in MDA231 cells transduced with the Ad-PR vector (MOI range: 500, 1000, 2500 and 5000) exposed to either normoxic (N, 21% O_2) or hypoxic (H, 0.1% O_2) conditions. (B) The potency of the Ad-PR vector is greater than the Ad-P vector in transduced CPA-treated MDA231 cells exposed to both normoxic (N) and hypoxic (H) conditions measured by BrdU incorporation (MOI=1000). The graph shows the mean % control (BrdU incorporation) ± SD of a representative experiment. (C) The potency of the Ad-PR vector is greater than the Ad-P vector in transduced LS174T cells exposed to CPA in both normoxia (N) and hypoxia (H) as measured by BrdU incorporation (MOI=1000). The graph shows the mean % control (BrdU incorporation) ± SD of a representative of 3 experiments.

than either vector alone at inhibiting the proliferation of MDA231 cells in the presence of CPA. For example, 0.05 mM CPA inhibited the proliferation of the MDA231 cells by more than 80% when they were transduced by both the Ad-R and Ad-P vectors compared to no inhibition with either vector alone.

A clonogenic survival assay was used to determine the impact of primary human macrophages transduced with the different adenoviral vectors on the colony forming ability of the T47D breast carcinoma cell line (Fig. 1C). In the presence of 1 mM CPA, macrophages transduced with both Ad-P and Ad-R vectors completely inhibited the colony formation by T47D cells compared to moderate inhibition by macrophages transduced with Ad-P or Ad-R vectors alone. These data confirm that overexpression of P450R can enhance the cytostatic effect of P450 in the presence of CPA.

In vitro evaluation of a recombinant adenovirus expressing both P450 and P450R. An adenoviral vector was designed and constructed to co-express human cytochrome *CYP2B6* and the human *P450R* genes [Fig. 1A (Ad-OBHRE-CYP2B6-CMVP450R, Ad-PR)]. In this vector the cytochrome P450 2B6 gene is regulated by the synthetic hypoxia responsive (OBHRE) promoter (19) to restrict the expression of this gene to the hypoxic regions of the tumour microenvironment. We employed 0.1% O_2 hypoxic incubation in the *in vitro*

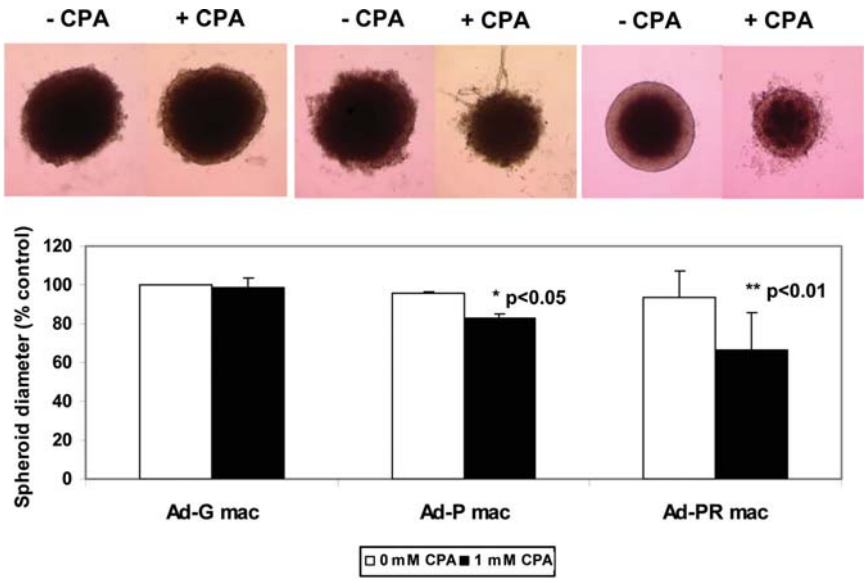


Figure 3. T47D tumour spheroids were incubated with macrophages transduced with the different adenoviral vectors as described in Materials and methods. Representative light microscope photographs of whole spheroids that were exposed to the different treatment regimens are shown in the top panel. Spheroid diameters were obtained by taking measurements from five T47D spheroids in each condition. The graph shows the mean % control (spheroid diameters of Ad-G mac exposed spheroids) ± SD from one representative of three experiments.

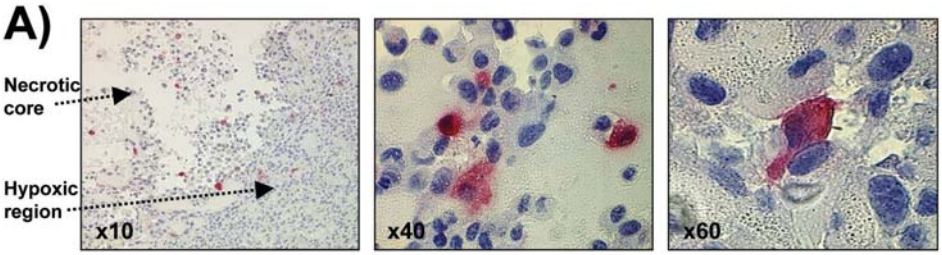
experiments because the direct measurement of oxygen tension in patient tumours showed a median oxygen tension range of 1.3-3.9% (10-30 mmHg) with the lowest reading at 0.01% (0.08 mmHg) (20). In this study, 82% of all readings taken in solid tumours were <0.33% O_2 (2.5 mmHg).

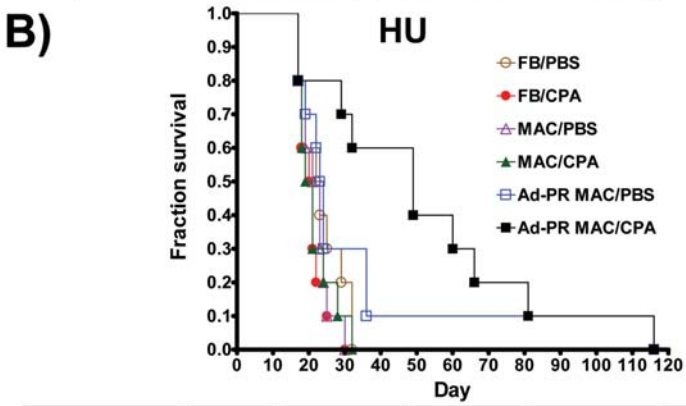
The expression of the P450 2B6 and P450R proteins in Ad-PR transduced MDA231 cells was examined using Western blotting. As expected, P450 2B6 was not expressed in the normoxic exposed cells but was induced by hypoxia (Fig. 2A). In contrast, P450R was constitutively expressed at high levels in the transduced cells under both normoxic and hypoxic conditions in a vector dose-dependent manner. The potency of the Ad-PR adenoviral vector was directly compared to that of the Ad-P vector in the MDA231 and LS174T tumour cell lines *in vitro* (Fig. 2B and C). The activity of P450 is enhanced by the P450R expression in MDA231 cells leading to a decrease in survival down to 3% in the presence of 1 mM CPA and hypoxia. The impact of hypoxia on cell survival was even more acute in the Ad-PR transduced LS174T cells with 1% survival following hypoxic exposure. Similar results were also obtained in the OVCAR-3 human ovarian cancer cell line (data not shown). Interestingly, there appeared to be some CPA-mediated cytotoxicity for Ad-PR vector transduced MDA231 cells under normoxia. This is partly due to the presence of some cytopathic effects of the Ad-PR vector transduction on these cells. In addition, MDA231 cells are fast-growing cells and there may be some small clusters of cells forming during the incubation period, leading to OBHRE promoter-driven CYP2B6 expression in these localized hypoxic areas.

Effect of CPA on T47D spheroids infiltrated with genetically modified primary human macrophages. The potency of the macrophages transduced with the Ad-PR vector was further investigated *in vitro* using the spheroid and macrophage co-culture system. Spheroids are three-dimensional multicellular aggregates that can be used to model the avascular tumour microenvironment (21). We have previously shown that incubation of the spheroids under hypoxic conditions induces the expression of P450 2B6 in infiltrated macrophages transduced with an Ad-OBHRE P450 2B6 vector (16). In this study we used macrophages transduced with either adenoviruses expressing the GFP reporter gene, Ad-G, or with the Ad-P vector as controls to compare with macrophages transduced with the Ad-PR vector. Transduced macrophages were co-cultured with T47D spheroids in hypoxic conditions in the presence or absence of CPA. In the presence of CPA, the diameter of the spheroid infiltrated with Ad-PR transduced macrophages decreased by 28% (Fig. 3, t-test $p<0.01$), whereas the diameter of the spheroid infiltrated with Ad-P transduced macrophages decreased by 13% ($p<0.05$). There was no significant change in diameter of the spheroids infiltrated by the Ad-G transduced macrophages ($p>0.05$).

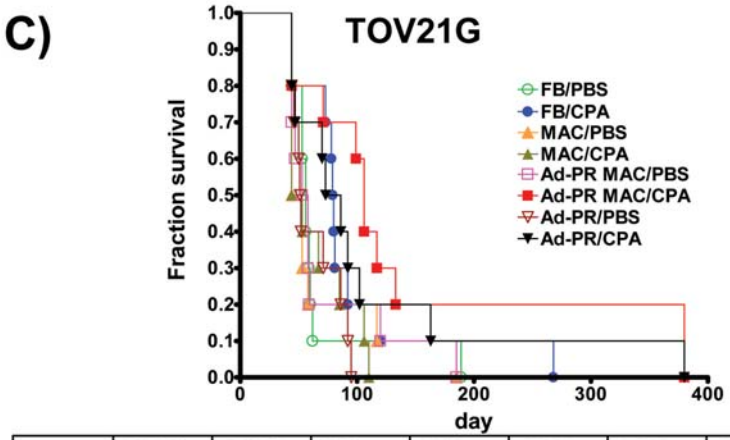
Evaluation of genetically engineered macrophages on survival in mouse HU and TOV21G tumour xenograft models. We investigated the impact of Ad-PR modified macrophages on survival in mouse tumour xenograft models. Initially, we wanted to determine that human macrophages could infiltrate tumour xenografts *in vivo*. Primary human macrophages transduced with the GFP expressing adenovirus (Ad-GFP) were administered peritumourally to mice bearing MDA231 tumour xenografts. Two days later the MDA231 tumours were harvested and GFP-expressing macrophages were detected histologically within the necrotic core and the surrounding hypoxic region of the tumour xenograft (Fig. 4A).

The therapeutic activities of the Ad-PR modified macrophages were subsequently evaluated in the HU and TOV21G human ovarian xenograft models that can be established i.p., mimicking the formation of ovarian tumours *in vivo*. In the





	FB/PBS	FB/CPA	MAC/PBS	MAC/CPA	Ad-PR MAC/PBS	Ad-PR MAC/CPA
Median survival	22.5	20.5	22	20	23.5	49



	FB/PBS	FB/CPA	MAC /PBS	MAC /CPA	Ad-PR MAC/ PBS	Ad-PR MAC/ CPA	Ad-PR /PBS	Ad-PR /CPA
Median survival	56	79.5	48.5	44	53	106	51.5	79.5

Figure 4. (A) A representative cross-section of MDA231 intraperitoneal tumour xenografts showing red GFP-positive human macrophages tranduced with Ad-G vector 48 h after peritumoral administration. (B) Ad-PR transduced macrophages plus CPA increase the survival of mice bearing the HU xenograft compared to control groups (FB, formulation buffer; PBS, phosphate buffered saline; MAC, untransduced macrophages, Ad-PR MAC, Ad-PR vector transduced macrophages (MOI=500)]. Each mouse was injected i.p. with either 0.2 ml of PBS or CPA (5 mg/ml) on days 17 and 19 and weekly thereafter. The table shows the median survival values obtained by the Kaplan-Meier survival analysis. (C) Ad-PR transduced macrophages plus CPA increase the survival of mice bearing TOV21G tumour xenografts compared to control groups. The table shows the median survival values obtained by the Kaplan-Meier survival analysis.

HU tumour xenograft study, a single dose of primary human macrophages transduced with the Ad-PR vector was injected i.p. followed by repeated CPA treatment. These mice showed increased survival (median survival, 49 days, Kaplan-Meier survival analysis, Fig. 4B) compared to the control groups including mice treated with formulation buffer and CPA (median survival, 20.5 days, $p<0.01$); untransduced macrophages and CPA (median survival, 20 days, $p<0.01$); or untransduced macrophages and PBS (median survival, 22 days, $p<0.01$) (Fig. 4B). However, despite a difference in the median survival of mice treated with Ad-PR transduced macrophages in the presence and absence of CPA (49 vs. 23.5 days, respectively), there was no statistical significance ($p=0.182$) between these two groups.

The same treatment strategy was used in the TOV21G tumour xenograft model with the addition of an Ad-PR vector only treatment group (Fig. 4C). In this study, the Ad-PR vector transduced macrophages plus CPA treatment group showed significant extended survival (median survival, 106 days) compared to the control groups including mice treated with formulation buffer only (median survival, 56 days, $p<0.01$); untransduced macrophages plus CPA (median

survival, 44 days, $p<0.01$); or macrophages transduced with the Ad-PR vector in the absence of CPA (median survival, 53 days, $p<0.01$). However, there was no statistical significance between the Ad-PR vector transduced macrophages plus CPA treatment group and the group treated with the Ad-PR vector only with CPA (median survival, 79.5 days, $p=0.14$). In addition, the median survival of mice treated with CPA alone (79.5 days) is significantly greater than that of formulation buffer-treated control mice (56 days, $p<0.01$) indicating that the TOV21G tumour is sensitive to CPA treatment. It also appeared that the untransduced macrophages with CPA combination (MAC/ CPA) shortened the median survival of CPA alone from 79.5 to 44 days ($p=0.12$) suggesting that untransduced macrophages might protect the tumour from CPA. This is in agreement with previous studies in which tumour associated macrophages have been shown to promote tumour growth (21).

Discussion

Our results suggest a benefit of developing a genetically engineered macrophage for the treatment of cancer. Particularly, it may be useful for the treatment of cancers that are confined to the abdomen, such as ovarian cancers, since macrophages are attracted to MCP-1 (now known as CCL2) produced by ovarian tumours and are present in significant numbers near the necrotic region where hypoxia down-regulates MCP-1 (6-8). The treatment strategy would typically involve patients undergoing apheresis to collect autologous monocytes, maturation to macrophages in the presence of GM-CSF, 'arming' of these macrophages via transduction with the Ad-PR vector and re-administration directly into the peritoneal cavity followed by an intraperitoneal infusion of CPA. The principle of this *ex vivo* approach has been shown to be safe and well-tolerated in human cancer clinical trials where at least $3x10^9$ patient-derived macrophages were activated with either (IFN-γ) or LPS prior to re-infusion (10,22). In these studies the macrophages remained in the peritoneal cavity for over 7 days and accumulated at tumour sites (10).

Using a retroviral based product called MetXia® the P450/CPA GDEPT approach has been shown to be safe and well-tolerated in human clinical trials (15,23). Primary human macrophages have been shown to up-regulate HIF under hypoxia (24) and we have previously shown that the OBHRE promoter restricts *lac*Z expression to the hypoxic region of tumour xenografts in adenovirally-transduced macrophages that have infiltrated tumour spheroids *in vitro* (16,17). Furthermore, we have shown that primary human macrophages express HIF-2 in response to hypoxia and can regulate the production of CYP2B6 driven by the OBHRE promoter (16). Therefore, we hypothesised that the combination of the OBHRE promoter regulating P450 expression and the tumour infiltrating properties of the macrophages should be able to limit the expression of the *CYP2B6* gene to the hypoxic regions of the tumour microenvironment, thus avoiding unwanted expression of P450 2B6 in normal tissues. The aim is to limit activation of the prodrug to the tumour thereby minimising the systemic effects of the activated prodrug. An additional safeguard of this strategy is that it naturally terminates with the death of the macrophage which has a lifespan of approximately three months (25). Furthermore, any undesirable adverse effects during treatment can be terminated by withdrawing the prodrug. This strategy also permits re-administration of genetically modified macrophages, should a repeat dose be required, more readily than a strategy based solely on adenoviral vectors where pre-exisiting anti-adenovirus antibodies or antibodies formed following the first treatment limit the second dose. The potency of this GDEPT strategy may be enhanced further by replacing the *CYP2B6* gene with, for example, the canine P450 2B11 isoform that has improved pharmacokinetics for CPA (26,27).

In summary, we have presented data to propose that macrophages may be used as a tumour-infiltrating carrier for a hypoxically regulated GDEPT strategy based on the *P450* and *P450R* genes to elicit bystander tumour cell killing in the presence of a prodrug such as CPA.

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