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Behaviour of transplanted tumours and role of matching in rejection

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ABSTRACT

Background: Tumour transfer/development is one of the more serious risks associated with transplantation. The behaviour of a tumour can be unpredictable in immunosuppressed recipients. We report a highly sensitive method to monitor tumour behaviour in real time in a rodent tumour transplant model. This paper also explores the effect of MHC matching on tumour growth among control and immunosuppressed hosts.
Methods: Luciferase expressing Wistar rat kidney tumour cells were transplanted into either Wistar or Lewis recipients which mimic a well and poorly matched combination to assess the effects of MHC matching on transplanted tumour cells. Experimental groups included controls with no immunosuppression and animals immunosuppressed with cyclosporine. The latter group was further divided into a continuous treatment group which received four weeks of immunosuppression and a treatment withdrawal group where immunosuppression was stopped after two weeks to assess the effects of rejection on tumour growth.
Results: All the tumour cells were rejected in the control animals that received no immunosuppression, within 2 weeks among well-matched combination and within one week in the poorly matched combination (p 0.001). The transplanted tumour cells continued to grow in both well-matched and poorly matched groups who were treated with cyclosporine, but growth was significantly faster in the well-matched combination (p 0.033). After treatment withdrawal the tumour cells were rejected in all the animals of the poorly matched group compared to 50% in well matched animals within the four-week study period (p 0.039).
Conclusion: In the absence of immunosuppression the hosts reject the transplanted tumour cells, and the anti-tumour response is stronger when there is a greater mismatch in MHC with the recipient. In the presence of cyclosporine immunosuppression the tumour continues to grow, however, after withdrawal of the immunosuppression, tumour clearance is quicker in the poorly matched background. This data supports the idea of expansion of the donor pool by using kidneys after ex vivo resection of small renal tumours and that these organs should be transplanted into a less well-matched HLA recipient. We hypothesise that should a tumour recurrence occur a poorly matched recipient could clear the tumour through withdrawal of immunosuppression.

1. Background

Transplantation has revolutionised the treatment of patients with renal failure. It not only improves quality of life but also has a significant survival advantage compared with dialysis [1]. Although graft survival and the absolute number of allografts have increased over the past couple of decades, there remains a large gap between the number of organs available and potential recipients [2]. Over the years new sources of organs have been explored but the problem persists and there is still a need to increase donor numbers.

There is a large body of evidence that patients with small renal cell carcinomas (RCC) can be treated with nephron sparing surgery (NSS) with comparable outcomes to the previous gold standard of radical nephrectomy [3,4]. Consequently for a patient electing to have their whole kidney removed for a small RCC there is a potential for the removal of the tumour and then allotransplantation of the remaining kidney. This approach has been utilised by a few groups with good results [5–9]. One of the most important and perhaps potentially dangerous differences between a urology patient that has undergone NSS for a small RCC and a potential allograft recipient of an NSS kidney is that transplant recipients are on lifelong immunosuppression. Immunosuppressive agents inhibit the natural checks on cancer cells by the immune system. It is not known how tumour cells will behave in a HLA incompatible immunosuppressed host, if there is any inadvertent transplantation along with such restored kidneys.

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In the absence of any immunosuppression the allograft is rejected. Theoretically any tumour cells transplanted along with the allograft should be rejected as they both originate from the same donor. However, cancerous cells have the ability to make themselves less immunogenic thereby evading the donor immune system in the first instance and it is not clear how they will behave in a new host [10].

2. Objectives

The aim of this study was to establish a rodent tumour transplant model and study the effects of immunosuppression on tumour growth. The other main aim was to study the effects of acute rejection on tumour cells in a transplantation setting.

3. Study design and methods

3.1. Cell culture

The tumour cell line, BP36b was acquired from Riken Bio Resource Centre (BRC) Cell Bank© Japan. This is a rat kidney tumour cell line derived from male Wistar rats that received N-ethyl-N-hydroxyethylnitrosamine (NHEN) in drinking water to induce tumour growth. The cell line is stable and maintained its characteristics after 100 passages over a 3 year period [11]. Cells were grown in RPMI 1640 supplemented with glutamine and antibiotics (penicillin 10,000 units ml^{-1}, streptomycin 10 mg ml^{-1}, gentamicin 50 μg ml^{-1} and amphotericin B 25 μg ml^{-1}). The doubling time of the cell line was consistent with the reported time in the literature (17 h) [11].

3.2. Transfection

For real time in vivo imaging of the tumour cells, the cell line was transfected with a commercial lentiviral construct that is stably integrated and constitutively expresses the enzyme luciferase for bioluminescence and green fluorescent protein (GFP) for florescence [12,13]. Puromycin (10 μg ml^{-1}) was used for selection of stable transfectants.

Puromycin supplemented media was replaced every 48–72 h to select for single colonies of stable transfectants. Transfectants were initially assessed by the expression of GFP by florescence microscopy. Bioluminescence was determined initially by a luminometer and then by direct visualisation using the IVIS® spectrum imaging system (Caliper Inc.) (Fig. 1).

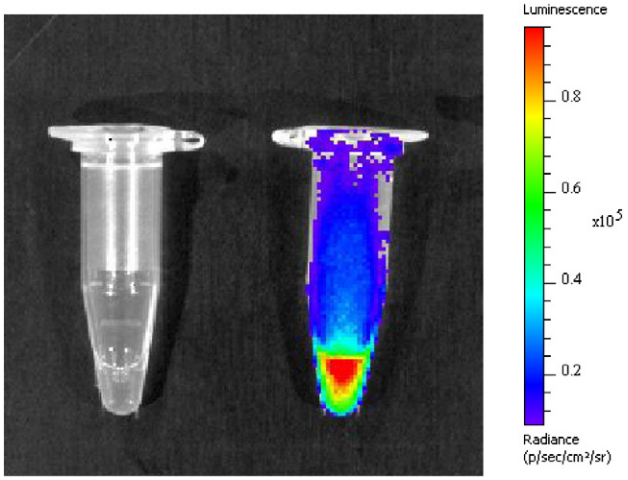

Fig. 1. IVIS spectrum image of non-transfected cells (left) and transfected cells (right). The system produces a heat map image that can be compared to the scale seen to the right of the image and the intensity of the luminescence calculated (P/s/cm^2/sr).

3.3. Tumour transplantation

Animals were injected with a fixed number of cells (1.8×10^7) into the right flank under Isoflurane anaesthesia after shaving the fur. The animals were anaesthetised in the induction chamber of the IVIS spectrum imaging system and then transferred into the dark chamber where they were scanned for varying lengths of time (60–300 s). Animals were kept anaesthetised in the imaging chamber to enable long exposure times required to detect even very faint bioluminescent signals. Luciferin was injected intraperitoneally at the dose of 150 mg/kg 10–15 min before scanning to allow circulatory distribution throughout the animal before detection. Timing of luciferin injection was calculated by plotting the kinetic curve prior to the experiments. To compensate for variations in luciferin distribution, 2–3 images were taken of each animal at different time points and the only image with the strongest signal used for further analysis. Regions of interest (ROIs) were the areas of cell injection and any other areas with positive signals. The background luminescence was calculated for each animal and signal intensity was calculated by subtracting this from the ROI value to get the accurate value of signals from the transplanted tumour cells (Fig. 2).

3.4. Experimental groups

To study the effects of matching on transplanted tumour growth two different strains of rats, Wistar and Lewis were used. Since tumour cells were of Wistar origin, when injected into Wistar rats (outbred) [14] this combination served as a well-matched group as both the animals were of the same strain. Despite the similarities between the tumour cell line and the recipients, these animals were not true syngeneic to the tumour cells due to being outbred [15]. The other group was of inbred Lewis animals that served as a poorly matched group due to transplantation across the strain, leading to more marked immunological differences.

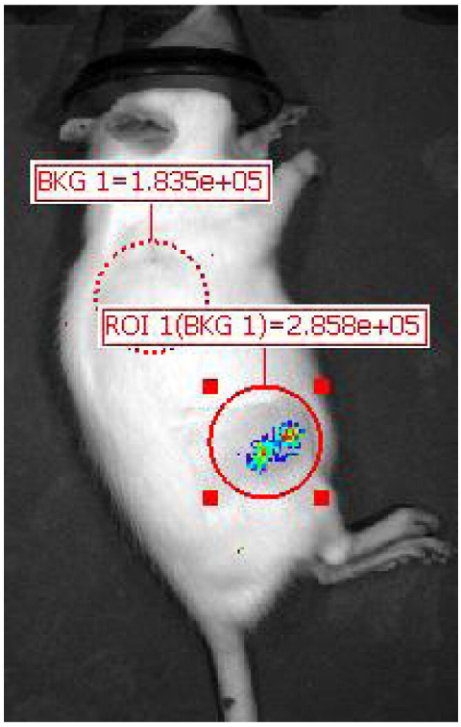

Fig. 2. Day 0 IVIS spectrum image of Wistar rat after injection of transfected tumour cells into the right flank. Imaging was performed 15 min after intra-peritoneal injection of luciferin for maximum signal intensity. Region of interest (ROI, solid red circle) is the area of positive signals from the injection site while the background bioluminescence (dotted red circle) is calculated for each image to calculate bioluminescence.