Intra-renal Transplantation of Bioactive Renal Cells Preserves Renal Functions and Extends Survival in the ZSF1 model of Progressive Diabetic Nephropathy

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ABSTRACT: There are >200,000 diabetic patients in the US with End-Stage Renal Disease. Hemodialysis and pharmacological intervention insufficiently support diminishing kidney function long term, culminating in whole kidney transplantation or death. New treatment paradigms that slow or reverse progression of chronic kidney disease (CKD) are needed to relieve patient and healthcare burdens. Recent work in our laboratory demonstrated that a selected population of bioactive renal cells (BRCs), established from autologous diseased kidney tissue, regenerated *in situ* functional kidney mass, stabilized filtration function, and prolonged survival following intra-renal delivery in a 5/6 nephrectomy model of terminal CKD[1]. In the present study, the *in vivo* function of these BRCs was evaluated in the ZSF1 rodent model of progressive nephropathy secondary to a metabolic syndrome of diabetes, obesity, dyslipidemia, and hypertension. Injection of syngeneic BRCs into the ZSF1 renal parenchyma elicited a regenerative response that significantly improved renal functions, including filtration (BUN, sCre, eGFR), protein handling (albumin), electrolyte balance (K, Na, Phos) and the ability to concentrate urine (osmolarity) confirming similar results observed in a severe mass reduction model of CKD[1]. Multivariable linear regression analysis showed that each of the renal functions affected by treatment significantly predicted ZSF1 survival beyond the one year timeline for follow up. The characteristic hypertension in the ZSF1 model was attenuated at 50 weeks of age by the cell treatment; these data were further supported by statistically significant modulation of physiological regulators of blood pressure, including the pressor hormones, ACTH, cortisol and renin. Also consistent with previous results, implantation of the BRCs in the ZSF1 model resulted in significant reduction of circulating plasminogen activator inhibitor (PAI), a master regulator of tissue fibrosis. These results collectively form the basis for justifying the clinical use for intra-renal delivery of an adult autologous and regenerative renal cell population for slowing disease progression in CKD patients secondary to metabolic syndrome.

METHODS: ZSF1 rats were purchased from the vendor (Charles River Labs, CRL) and delivered to a CRL Division of Discovery Services, Piedmont Research Center (Morrisville, NC), housed and monitored for 8 weeks prior to treatment. All procedures were conducted in accordance with NIH and IACUC guidelines. A 45 week time course evaluating the syngeneic implantation of bioactive renal cells was investigated in male obese ZSF1 rats. After the establishment of disease (i.e. persistent proteinuria), rats were randomized to the obese ZSF1 test treatment group (Obese Tx, n=5) or the untreated obese ZSF1 control group (Obese, n=5). Renal cell treatment (18 weeks of age) was followed by standard of care measures for controlling hyperglycemia (switch from diabetogenic to healthy diet + insulin). Kidney cell fractions were isolated from the primary culture established from adult female ZSF-1 (6-8 week old) kidneys using established protocols[1]. The animals were anesthetized and the kidneys were exposed via a retro-peritoneum approach through a dorso-lateral incision. BRCs were delivered into the right and left kidney parenchyma, accessed via the cranial and caudal poles of each kidney, using a 23 gauge needle. A cell dose of 3.0 million cells in 100 µl sterile PBS was administered bilaterally to each kidney pole for a total dose of 6.0 million cells/~2.5 g kidney. The suspended cells were loaded into a 1-ml syringe fitted with a ½-inch 23-gauge needle and delivered directly to the kidney through the apical cortex at a depth of approximately 5 mm. The animals were followed post-operatively using in-life assessment that included serum chemistry, hematology, urinalysis, blood pressure and weight gain. End-of-life assessment included organ weights, histology and survival. Systolic and diastolic blood pressure (BP) were measured and mean arterial BP (MABP) was calculated on a monthly schedule using a CODA non-invasive tail-cuff BP monitor (Kent Scientific). PAI-1 and ACTH were measured using Rules Based Medicine (RBM, Austin, Tx) assays. Renin was measured using an Uscn Life Sciences Inc. ELISA kit (Wuhan, China). Graphing and statistical measures were performed with JMP version 7.0 from SAS Institute Inc. (Cary, NC).

RESULTS

Figure 1.Prolonged Survival

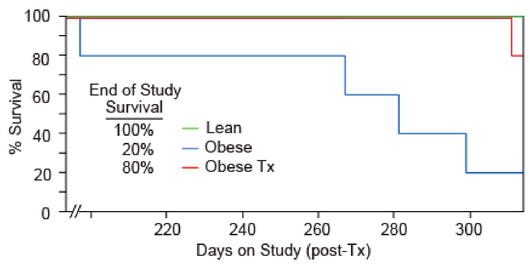


Figure 1. Survival advantage with Male Obese ZSF1 rats treated with Bioactive Renal Cells (BRCs). Lean, littermate controls (green, n=5) survive the duration of the study with early signs of disease progression. Obese littermate controls that were treated with BRCs (red, n=5) out-survived their Obese littermate control counterparts (blue, n=5).

Figure 2. Improved Renal Function

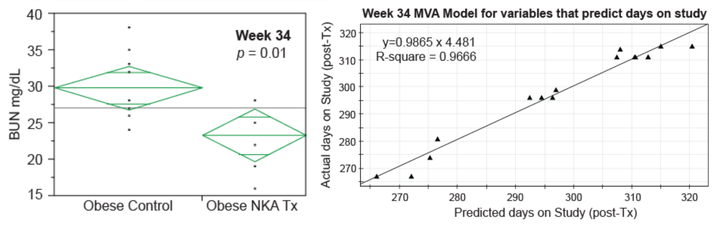


Figure 2. Renal Function was assessed serologically and through urinalysis. A Multi-variate model analysis (MVA) for parameters that predict days on study was performed. Using Week 34 data as an example, BUN (shown in right panel) along with Cholesterol, serum Albumin, sodium, potassium, eGFR and urinary Specific Gravity significantly predicted survival.

Figure 3. Composite Function

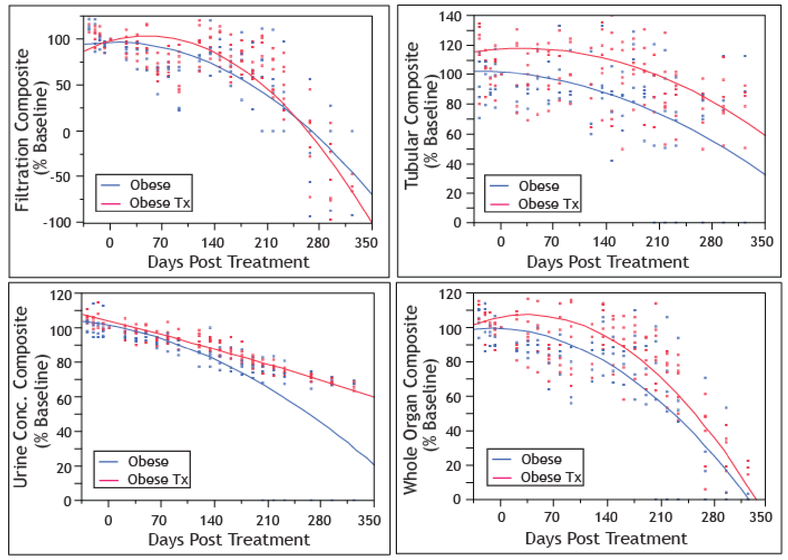


Figure 3. Composite measurements as a function of the major renal compartments and representing whole organ function. Statistically significant differences in serum and urine renal function measurements are calculated uniformly on the same 100% scale as % of baseline to give each measurement equal weighting; composite scores were fitted to bivariate curves using a 2nd order polynomial. The Filtration Composite collectively measured BUN, serum creatinine, cholesterol, and urinary creatinine. The Tubular Composite collectively measured total serum protein, serum calcium, urinary sodium, calcium and phosphorous. The urine concentration composite collectively measured urine osmolarity and specific gravity.

Figure 4. Composite to predict survival

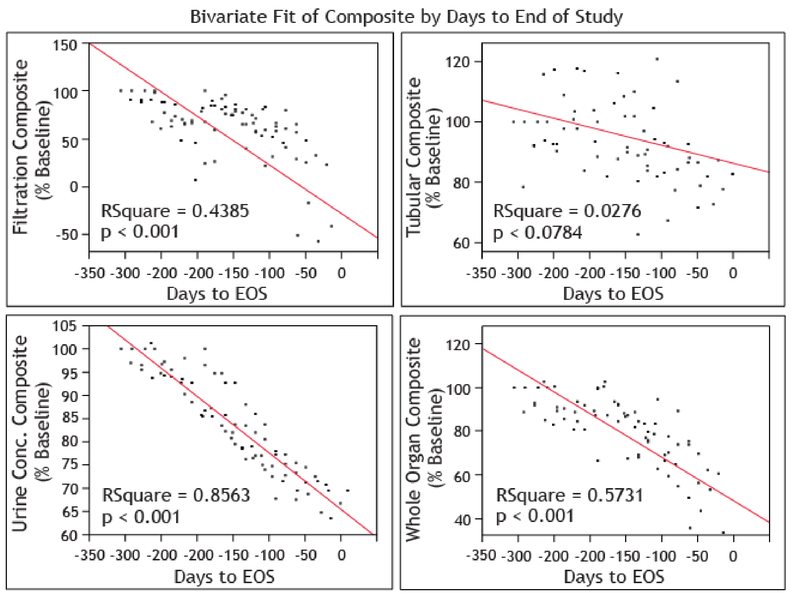
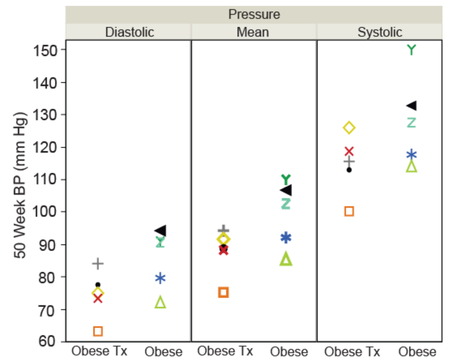


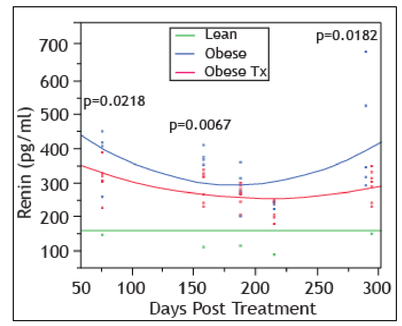
Figure 4. Linear bivariate fits of composite renal compartment and whole organ measurements significantly predict days to end of study (EOS). Data compiled from all animals (Obese and Obese Tx) that died prior to the end of study (Day 315). Of the collective filtration, tubular or urine concentration measurements (Fig. 3), a composite reflecting the ability of the nephron to concentrate urine (uOSMO & uSG) and the whole organ composite (all measurements represented in Fig. 3) most significantly predicts survival.

References cited:

1. Kelley et al., 2010 AJPRenal Physiol 299 (5).

Figure 5. Improved Blood Pressure





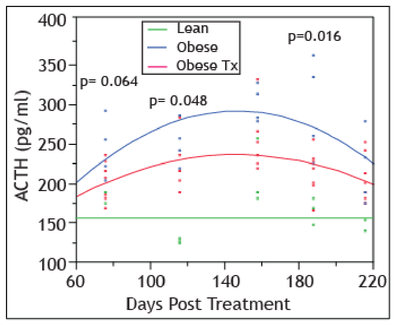


Figure 5. Impact of BRC-treatment on Arterial Blood Pressure and physiological regulators of BP. ZSF1 rats treated with BRCs showed a significant trend in reductions to diastolic, systolic and mean arterial blood pressure (MABP; $P = 0.07$). These data were supported by significant Immuno-detection improvements to serum Renin (pg/ml) and ACTH (pg/ml). Lean measurements (green) are presented linearly as the average of all lean time points; Obese and Obese Tx measurements are bivariate fits using second order polynomial equations. NKA improved blood pressure by affecting both the target organ (juxtaglomerular apparatus) and sympathetic activity (pituitary).

Figure 6. Attenuation of circulating PAI-1

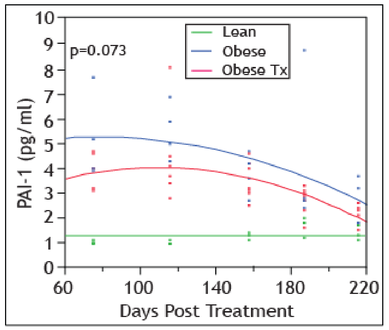


Figure 6. Reduction in a master regulator of fibrosis, PAI. Consistent with previously published data[1], a trend towards significant reduction in the serum plasminogen activator inhibitor-1 (PAI-1) following Implantation of BRCs in the ZSF1 model. Analysis are ongoing for end of study tissue histopathology and measures of PAI-1 and TGF-β mediated deposition of ECM.

CONCLUSIONS

In the present study therapeutically-relevant bioactive renal cells improve a myriad of renal functions and extend survival in a terminal model of progressive nephropathy secondary to a metabolic syndrome of diabetes, obesity, dyslipidemia, and hypertension:

- Multiple parameters of renal function were improved following BRC treatment, including compartmental and whole organ composite measurements
- Parameters of filtration, tubular and collecting duct function, along with whole organ composite measurements significantly predicted ZSF1 survival beyond the one year timeline for follow up
- BRC implantation improved MABP and significantly improved physiological regulators of BP, including the sympathetic pressor hormone, ACTH and the renal-specific hormone, Renin
- Reduction of circulating plasminogen activator inhibitor (PAI-1) is consistent with the reduction in renal inflammation both in the present study (data not shown) and in a previously published report[1]
- That BRC treatment significantly stabilized urine concentrating ability of the nephron is a functional indicator of attenuated interstitial disease and is consistent with reduced circulating PAI-1