The Role of Akt1 in Ovarian Graft Reception

Yoni Cohen[1], Hagit Dafni[1], Inbal Biton[1], Tal Raz[1], and Michal Neeman[1]
[1]*Weizmann Institute of Science, Rehovot, Israel*

Objective:

Ovarian tissue cryopreservation and transplantation is one of few available treatments for fertility preservation in women receiving chemotherapy or radiation therapy. In these patients the risk for ovarian failure can reach 92%, posing great risk for their childbearing potential. Ovarian tissue is retrieved and cryopreserved prior to initiation of chemotherapy. Successful ovarian tissue transplantation depends on rapid re-establishment and maintenance of blood perfusion. Akt1 knockout was associated with enhanced short-term angiogenic response and impairment of long-term blood vessel maturation. We used dynamic contrast enhanced magnetic resonance imaging (MRI) to explore the role of the PI3K-Akt1 pathway - a principle mediator of angiogenesis, on ovarian graft reception.

Materials & Methods:

Ovaries from 6 weeks old Akt1 knockout mice (Akt $^{-/-}$), heterozygotes (Akt1 $^{+/-}$) and wild types (Akt1 $^{+/+}$), were transplanted into the gluteus superficialis muscle of immunocompromised CD-1 female mice. Grafts were serially scanned on a 9.4 T MRI scanner 7, 14, 30 and 60 days after transplantation. Graft perfusion and angiogenesis were analyzed by dynamic contrast enhanced MRI analysis and computerized pixel by pixel analysis, generating concentration maps of biotin-BSA-GdDTPA for selected slices. Perfusion and angiogenesis were assessed by: 1). Blood volume fraction (fBV): The ratio between the extrapolated concentration of biotin-BSA-GdDTPA at the time of administration in the graft and the concentration in the blood; 2). Permeability of newly formed blood vessels (PS) was quantified by the initial rate of contrast accumulation in the graft, normalized to initial blood concentration. Data was analyzed using repeated measures ANOVA and Kruskall-Wallis non-parametric one-way ANOVA.

Results:

On day 60 mean calculated micro vascular density was significantly higher in Akt1 $^{+/+}$ transplants compared to Akt1 $^{-/-}$, 3.57% vs. 1.26% (p=0.0011) and there was a significantly higher mean PS in Akt1 $^{+/+}$ compared to Akt1 $^{-/-}$ transplants, 0.002 vs. 0.0007 min^{-1} (p= 0.0127) (figure 1). Mean vascular density and permeability in Akt1 $^{+/-}$ were not significantly different from Akt1 $^{+/+}$ or Akt1 $^{-/-}$. Furthermore, our in vivo MRI kinetic model enabled us to diagnose complete loss of graft perfusion and graft destruction by defining threshold levels of micro vascular density and permeability, below which the graft is lost (figure 2). Higher rates of graft loss on day 60 were found in Akt1 $^{-/-}$ grafts (3/7, 42%) compared to Akt1$^{+/-}$ (1/4, 25%) and Akt1 $^{+/+}$ (0/5, 0%) (figure 3).

Conclusion

Ovarian grafts lacking Akt1 activity fail to maintain long-term angiogenesis and perfusion and exhibit higher loss rates. The results of this study indicate that Akt1 signaling transduction is crucial for successful ovarian graft reception.

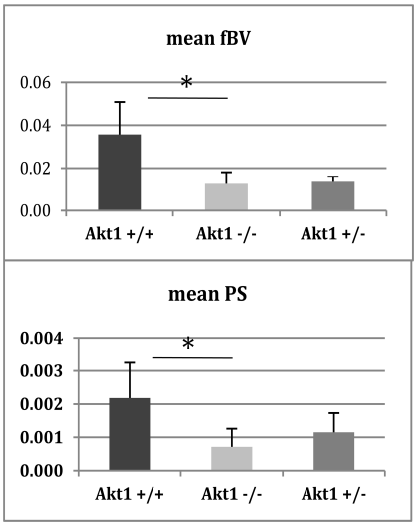

Figure 1. Quantification of microvascular density (fBV) and permeability (PS) 60 days after transplantation. Asterisks denote a significant difference between the Akt1 $^{+/+}$ and Akt1 $^{-/-}$ ovarian grafts.

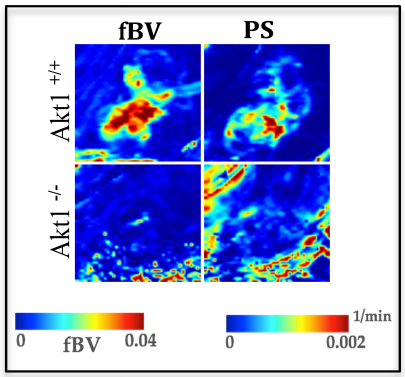

Figure 2. Blood volume and vascular permeability in ovarian grafts. 60 days after transplantation, showing Akt1$^{-/-}$ ovarian graft loss.

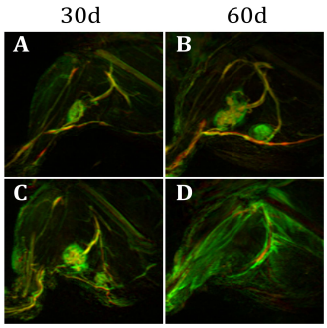

Figure 3. overlay of maximal intensity projections, showing loss of Akt1$^{-/-}$ ovarian graft within 60d after transplantation. **A-B**) Akt1$^{+/+}$ ovarian graft, **C-D**) Akt1$^{-/-}$ ovarian graft. Red and green color channels for early and late scans, 3 min and 40 min after contrast agent injection, respectively.