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Imaging of Qa-2 Protein, the *Ped* Gene Product, and its Localization to Lipid Rafts on the Surface of Preimplantation Mouse Embryos

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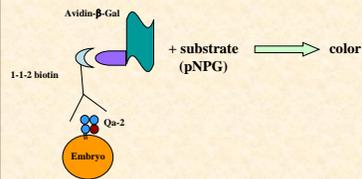


Abstract

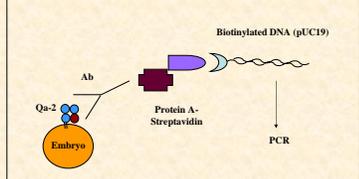
Mouse embryos that express the protein product of the preimplantation embryo development (*Ped*) gene, Qa-2, on their surface have been shown to cleave at a faster rate than Qa-2 negative embryos. Qa-2 positive embryos exhibit improved vitality throughout their development compared to Qa-2 negative embryos. In addition, offspring resulting from Qa-2 positive embryos are healthier as adults than offspring from Qa-2 negative embryos. The importance of Qa-2 protein in mediating embryo cleavage rate, embryo survival, and adult health has motivated us to develop methods to begin to understand the molecular mechanisms mediating Qa-2 action. As a first step, we have examined the location of Qa-2 protein on the embryo cell surface. Although it has been possible to detect membrane-bound Qa-2 molecules on preimplantation embryos using indirect methods, their low abundance on embryos has made previous direct visualization attempts unsuccessful. This poster presents the first successful use of new immunofluorescence reagents to visualize Qa-2 on mouse preimplantation embryos. We also have shown that Qa-2 protein is located in lipid rafts on the embryo cell surface. This finding suggests that Qa-2 acts as a signaling molecule in regulating the rate of preimplantation embryo cleavage divisions.

Materials and Methods

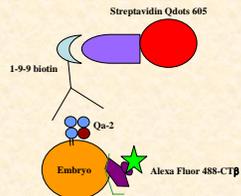
Embryo ELISA:



Immuno-PCR:



Immunofluorescence Imaging:

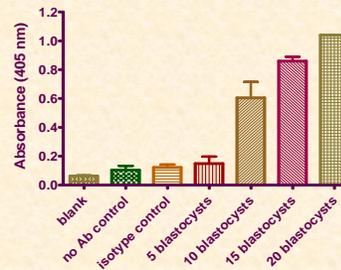


State of the Art

- Qa-2 protein is an important mediator of embryo survival.
- Qa-2 is expressed by preimplantation embryos^{1,2}, but has not previously been visualized on the embryo surface.
- Determining the location of Qa-2 on the membrane will allow insight into its distribution and mechanism of action³, both of which are unknown.
- Two indirect techniques (ELISA and I-PCR) have shown that Qa-2 is present on embryos, but the amount of Qa-2 cannot be determined without direct imaging.
- This poster presents the first successful direct imaging of Qa-2 on preimplantation embryos using new immunofluorescence reagents.

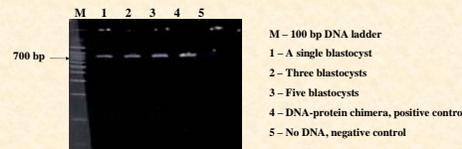
Results

ELISA on Embryos



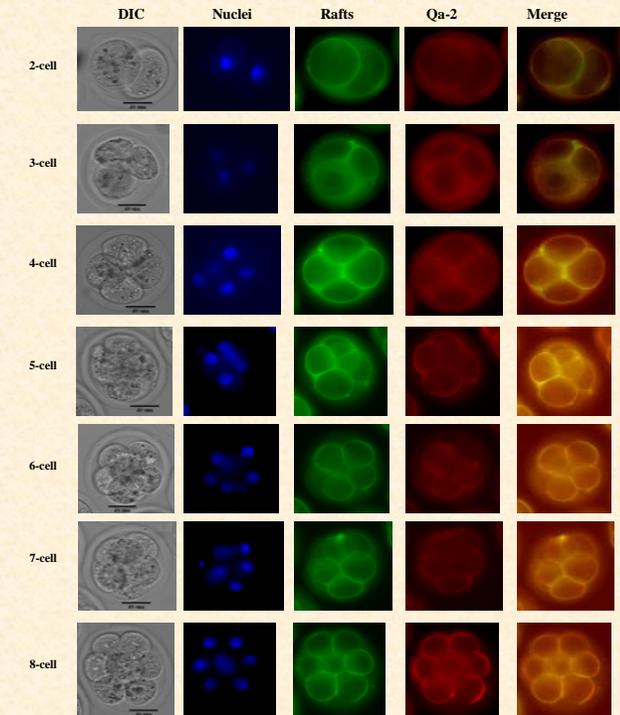
- Minimum of 5 embryos required for detection. The color intensity increases with more embryos in sample.

Immuno-PCR on Embryos



- A highly sensitive technique.
- Allows detection of molecules on a single embryo.

Imaging Qa-2 and its Localization to Lipid Rafts



Discussion and Future plans

- Techniques used confirmed the presence of membrane-bound Qa-2 on embryos, and its localization to lipid rafts.
- Visualization of Qa-2 on embryos will enable better understanding of its function.
- Visualization of Qa-2 will allow future imaging of the interaction of Qa-2 with signaling partners.

References

1. McElhinny AS, Warner CM. Detection of major histocompatibility complex class I antigens on the surface of a single murine blastocyst by immuno-PCR. *Biotechniques* 1997; 23:660-662.

Publications acknowledging NSF support:

2. Comiskey M, Goldstein CY, De Fazio SR, Mammolenti M, Newmark JA, Warner CM. Evidence that HLA-G is the functional homolog of mouse Qa-2, the *Ped* gene product. *Human Immunology* 2003; 64(11):999-1004.

3. Comiskey M, Domino KE, Warner CM. HLA-G is found in lipid rafts and can act as a signaling molecule. *Human Immunology* 2007; 68(1): 1-11.