CELL PROLIFERATION CONTROL AND THE ORIGIN OF NEOPLASIA IN THE GENOMICS AND PROTEOMICS ERA

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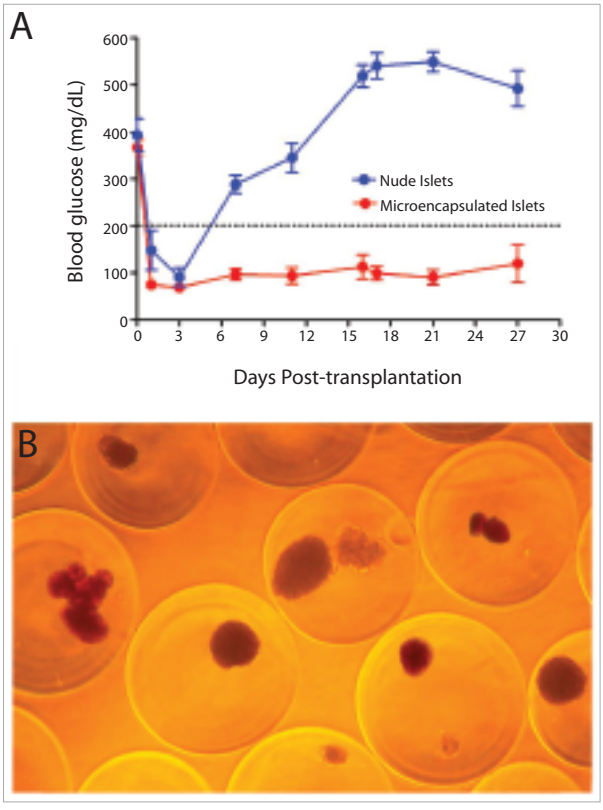


A: Reversion of diabetes induced by streptozotocin injection in immunocompetent Balb/c mice by microencapsulated human islets, shown in B

In the past two decades, the Cell and Molecular Biology Lab evolved to establish the Human Pancreatic Islet Unit and the Cell and Molecular Therapy Center (NUCEL – http://www.nucel.prp.usp.br), a center for translational research.

We focused mainly on cancer or the molecular basis for neoplasia, mainly, brain tumor (astrocytomas), prostate carcinoma, insulinoma and mammary carcinoma, by using state of the art technology to isolate and characterize genes which are differentially expressed in tumoral versus normal tissue. A number of collaborations were established with the medical community to allow access to patients samples, and new Bioinformatic tools were generated to deal with data obtained from using high throughput methods.

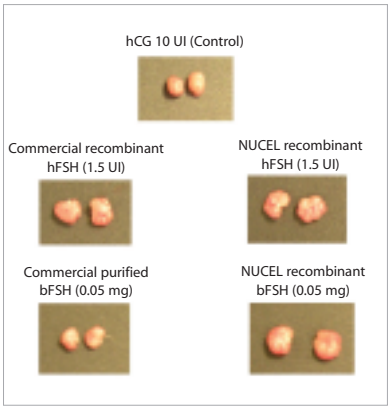
The group was also called upon to tackle another disease, namely, Diabetes mellitus (DM), which has been growing at a startling rate in the past few years, becoming a serious public health problem. Worlwide, around 10% of the almost 200 million diabetic patients are insulin-dependent (DM1), 5% of which are hyperlabile, with glycemic levels ranging from 50 to 2,000 mg/dL, which is seriously life-threatening. In 2002, our team introduced in Brazil an alternative treatment for these patients by means of pancreatic islet transplantation. However, since the islets are isolated from organ donors, this allotransplant requires immunosuppressive drugs, which cause secondary effects. We sought to establish a pre-clinical animal model for reversion of diabetes induced by streptozotocin, by using micro-encapsulated islets, and modifying the biomaterial used for encapsulation to improve islet survival and proliferation.

Organ shortage, relatively low islet yields and the need for a large number of insulin-producing cells to revert diabetes, prompted us to attempt to differentiate stem cells into insulin producing cells. Both murine and human embryonic and adult stem cells from different sources (umbilical cord, bone marrow, skin and dental pulp) are being used.

The ability to produce recombinant proteins allowed us to generate several products with great potential to become biopharmaceuticals, which are being transferred to the private sector for scaling up and commercialization.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Glioblastomas (GBM) are the most fatal tumors of the Central Nervous System, inefficiently treated with glucocorticoids (GC) chemotherapy. The quest for molecular markers for glioblastomas led us to use subtractive hybridization, DNA microarrays and Bioinformatics in a murine model system derived from the rat C6 glioma cell line, to isolate genes which are differentially expressed in ST1 and P7 rat C6 glioma variants, since the former is highly sensitive to GC while the latter is resistant to these hormones. DNA microarrays and Bioinformatics allowed us to identify a number of GBM markers, which may be used to generate DNA chips for diagnostics/ prognostics and new targets for gene therapy. New tumor markers for prostate carcinoma, insulinoma and mammary carcinoma were also identified.



In vivo *biological activity of human and bovine FSH (Follicle Stimulating Hormone) produced at the Cell and Molecular Therapy Center (NUCEL) compared to commercially available preparations*

We successfully established the animal model for diabetes and were able to cure this disease by injecting encapsulated human pancreatic islets into immunocompetent mice (Fig. 1). Cell Biology studies allowed us to propose new biomaterials for encapsulation of islets and other cell types. We were able to differentiate embryonic stem cells into insulin-producing, β-like cells, thus opening new avenues in diabetes treatment.

A number of cell lines overexpressing recombinant proteins were generated, which yielded products of biotechnological interest. For the first time in the literature, we obtained recombinant amylin/IAPP and its analogues, which are being used as insulin adjuvant to treat diabetes. Recombinant human and bovine FSH (follicle stimulating hormone), produced in high yields (Fig. 2), may be used for *in vitro* fertilization and animal management. Bone morphogenetic proteins (BMPs) 2 and 7, important inducers of bone regeneration, were produced and validated in pre-clinical trials. Secreted clotting factors VIII and IX were obtained and shown to display high biological activity *in vitro*. These and other products have attracted a number of biotech and pharmaceutical companies interested in transforming them in biopharmaceuticals.

In order to continue to pursue this translational research, the Cell and Molecular Therapy Center is forming new leaderships and building its own plant – a three-story building (1,600m^2) at the University Hospital area.

MAIN PUBLICATIONS

Colin C, Demasi MA, Degaki TL, Bustos-Valenzuela JC, Figueira RCS, Montor WR, Cruz LO, Lojudice FH, Muras AG, Winnischofer SMB, Hasegawa APG, Carreira AC, Verbisck NV, Granjeiro JM, Sogayar MC. 2008. NUCEL (Cell and Molecular Therapy Center) a multidisciplinary center for translational research in Brazil. *Molecular Biotechnology*. **39**: 89-95. Review.

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