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Development and characterization of 3 dimensional in vitro model of neuroendocrine tumors

Lilla Krokker^{1,2}, Borbála Szabó^{1,3}, Kinga Németh¹,
Báldzs Sarkadi³, Katalin Mészáros¹, Attila Patócs^{1,2},
Henriett Butz^{1,2}

¹ Hereditary Endocrine Tumours Research Group, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

² Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary

³ 2nd Department of Internal Medicine, Semmelweis University, Budapest, Hungary

⁴ Molecular Medicine Research Group, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

Introduction: In vitro two dimensional cell cultures are widely applied in cancer biology. However, there is a considerable difference among tumour cell behaviour of existing models and solid tumours because monolayer cell cultures do not represent the heterogeneity of human tumours reliably. Treatment options of endocrine tumours are limited and do not respond to radiation therapy either. Until now the information about 3D culturing of endocrine tumours is minimal.

Aims: Establishment of three-dimensional in vitro models of endocrine tumours and their comparison with monolayer cell cultures in order to identify novel pathomechanisms and potential new therapeutic targets.

Materials and methods: Induction of spheroid formation of pituitary tumours (GH3, RC-4B/C), adrenal carcinoma (H295R) and pheochromocytoma (PC12) cell lines was performed by different methods: using serum-free defined medium, ultra-low attachment plate and matrigel. AlamarBlue assay was used to examine cell viability and proliferation. Live and dead cell ratios were determined by trypan blue exclusion assay. The effect of mitotane treatment was assessed on H295R adrenocortical cells. Steroid hormone production was monitored by liquid chromatography coupled with tandem mass spectrometry. Inhibition of the SDHB enzyme in PC12 cells was done by itaconate and the glutaminase enzyme was inhibited by a selective inhibitor compound.

Results: While pituitary tumour cells were not able to form spheroids, H295R cells were capable of producing 3D structure using several conditions. Regarding PC12 cells spheroid induction was successful using SFDM. In 3D cultures H295R and PC12 cells further proliferated and kept their viability up to 10 days. H295R spheroid cell culture showed a higher tendency for cortisol production compared to monolayer cell culture. Significantly reduced hormone production capacity was identified after anti-proliferative effect of mitotane in spheroids. Regarding PC12 cells, the proliferation and viability upon itaconate and BPTES treatment of 3D and monolayer cultures showed difference.

Conclusions: Cell viability, growth characteristics and hormone production differed in 3D and 2D cultures and they showed different response to therapy as well. In vitro 3D models could be a useful model for neuroendocrine tumours mimicking solid tumour cell behaviour more reliably.

Supervisor: Henriett Butz

E-mail: butz.henriett@med.semmelweis-univ.hu

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Multi-organ damage following perinatal asphyxia in rat model

Tamás Lakat^{1,2}, A. Hosszu^{1,2}, A. Molnár¹, A. R. Toth¹,
K. Demeter⁶, H. Kelemen^{2,3}, E. Mikics³, A. J. Szabo^{2,4},
A. Dénes⁵, A. Fekete^{1,2}

¹ MTA-SE "Lendület" Diabetes Research Group, Budapest, Hungary

² Semmelweis University 1st Department of Pediatrics, Budapest, Hungary

³ Laboratory of Translational Behavioural Neuroscience, Institute of Experimental Medicine, Budapest, Hungary

⁴ MTA-SE Pediatrics and Nephrology Research Group, Budapest, Hungary

⁵ Laboratory of Neuroimmunology, Institute of Experimental Medicine, Budapest, Hungary

⁶ Behavioral Studies Unit, Institute of Experimental Medicine, Budapest, Hungary

Introduction: Perinatal asphyxia (PA) leads to death of more than half million mature newborns yearly. PA is associated with several serious complications including hypoxic encephalopathy, renal-hepatic- and cardiovascular injury, as well as respiratory distress. Basic research and clinical trials mainly focus on mitigating central nervous system damage by selective head or whole-body cooling. However, the extent of PA-associated multi-organ damage is not clarified yet and effective therapies are lacking.

Aims: The aim of the present study was to determine acute renal, hepatic and cardiac damage after PA and to identify pathways involved in the pathomechanism.

Methods: Postnatal 7 day-old male Wistar rat pups (n=5-10/group) were randomly grouped as follows: (i) Baseline: sacrificed immediately (ii) Control: pups were separated from the dam and incubated in normal air; (iii) PA: pups were separated and incubated in 4% O₂; 20% CO₂ in N₂ gas mixture for 15 minutes. Samples were collected after 4 (T4) and 24 (T24) hours. Serum levels of electrolytes, kidney and liver functional parameters and Troponin I were determined. Sensitive tubular injury markers Kim1 and Ngal were measured. Expressions of hypoxic (Hif1a, Hif2a), inflammatory (Il1a, Il1b, Il6, Tnfα), apoptotic (Bax, Bcl-2) and angiogenic genes (Vegf, Epo) and heat shock proteins (Hsp27, Hsp72) were investigated. Periodic-Acid Schiff and anti-CD45 staining on kidney, and Hematoxylin&Eosin staining on liver sections were performed.

Results: Blood urea nitrogen (BUN) and serum GPT were elevated at T4 following PA. Kim1, Ngal and heat shock protein expressions were increased, inflammatory and angiogenic pathways were activated in the kidney after PA. Vacuolisation, cytoplasmic degradation, and the onset of necrosis were observed in the liver following PA. In the liver hypoxic and apoptotic pathways were activated at T24 in the PA and in the control group. Serum Troponin I was elevated after PA indicating myocardial damage. Inflammatory cytokine and heat shock protein expression increased in the heart as well.

Conclusions: Acute renal, hepatic and myocardial damage was observed after PA. These results may justify the need for clinical follow-up and novel treatment strategies for possible multi-organ damage. The molecular pathways described here are potential targets for therapeutic intervention.

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Supervisor: Andrea Fekete

E-mail: andrea.fekete@gmail.com