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regulates expression of various genes that mediate cellular response to xenobiotics. The exact functional role of two AHR single nucleotide polymorphisms (SNPs); Arginine554Lysine (Arg554Lys) and Valine570Isoleucine (Val570Ile) has not yet been established, however studies suggest that these mutations might increase risk of developing PAs. To date, functional analysis of regarding the significance of these AHR SNPs in pituitary pathophysiology has never been analysed.

Aims

- Elucidate the effect of wildtype and polymorphic AHR on GH3 cell proliferation and on AHR-transcriptional response in the presence and absence of TCDD.
- Determine the allele frequency of the most common AHR SNP; the Arg554Lys in PA patients and in a small cohort of the Maltese population.

Method

The two missense mutations were introduced within the AHR-expressing vector and transfected in GH3 cells by magnetofaction, followed by the exposure to TCDD. Cell viability of GH3 transfected cells was measured using the MTT assay. Functional analysis of GH3 transfected cells treated with TCDD was carried out using luciferase assay and real-time PCR to detect and quantify the AHR-transcriptional activity. Genotyping of the Arg554Lys was performed on PA patients and neonatal controls using allele specific PCR. The Mann-Whitney test was used to compare two groups and Kruskall-Wallis test was used to compare three groups or more.

Results

In the absence and presence of low TCDD concentrations (1 and 10 nM), overexpression of wildtype AHR (wtAHR) did not affect GH3 cell proliferation. GH3 cells transfected with the AHR mutants did not exhibit any significant differences in their proliferative ability when compared with the wtAHR, both in the presence and absence of TCDD. Luciferase reporter analysis showed that there was a significant difference between the treated and untreated wtAHR ($P=0.016$), however this difference was not observed between the treated and untreated AHR mutants. Statistically significant difference in *Cyp1a1* gene expression analysis was detected between the treated and untreated wtAHR ($P=0.021$), Arg554Lys ($P=0.005$) and Val570Ile ($P=0.054$). Genotyping of the Arg554Lys in patients with PA gave a minor allele frequency (MAF) of 3% vs 0% in neonatal controls.

Conclusion

Gene expression and quantification analyses of AHR-target genes suggests that these AHR mutants might interfere with AHR target gene expression. Genotyping results suggested that this mutation is quite rare and may be similar to the frequencies of other European populations.

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P778

Comparative differential effects of secretagogues upon regulation of pituitary GH in several vertebrates

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It is known that the synthesis and release of pituitary GH is controlled by complex neuroendocrine mechanisms that involve several neuropeptides, such as GHRH, SST, PACAP, TRH, GnRH, Ghrelin, among other regulators. Previous reports indicate that, during vertebrate evolution, the potency and efficacy of these secretagogues may vary and play differential effects upon GH regulation. In this work we aimed to study, *in vitro*, the capacity of these peptides to control the expression and secretion of pituitary GH in three vertebrate models: rat (mammals), chicken (birds) and iguana (reptiles), employing pituitary cultures at different incubation periods (0–6 h) and two doses of the secretagogues (1 and 10 nM). Results showed that GHRH significantly stimulated GH mRNA expression as well as GH secretion in the three species within the first hour of incubation, in comparison to the controls. However, its effect upon GH mRNA was 60 times greater in iguana than in the other species. TRH had no effect on GH secretion in any incubation period, but it stimulated GH mRNA expression in all species and, in the case of iguana, its effect was 150 times higher than in the others. PACAP stimulated GH mRNA expression at 4 h in chicken pituitary cultures, whereas no significant differences were observed in rats and iguanas. Ghrelin increased GH secretion in chickens, but had no effect in its mRNA synthesis, contrary to what was found in iguana cultures where GH mRNA significantly diminished. GnRH stimulated both GH mRNA expression and GH release in chicken pituitary cultures, while in iguana only GH secretion was significantly increased. On the other hand, SST strongly inhibited GH mRNA expression and GH release in the iguana, while no significant effect was directly observed in rats and chickens, at the doses and time-frame conditions employed. Results indicate that there is a differential effect of these secretagogues upon GH synthesis and secretion during vertebrate evolution, and further studies are needed to understand how these mechanisms have evolved.

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P779

Next generation sequencing for characterization of mitochondrial genome in pituitary adenomas

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Introduction

Disrupted mitochondrial functions and genetic variations of mitochondrial DNA (mtDNA) have been observed in different tumors. Regarding pituitary adenomas mtDNA was evaluated only in oncocytic type using PCR based methods and it showed high prevalence of Complex I variants. Next generation sequencing (NGS) allows high throughput sequencing and it is useful for accurate identification of heteroplasmy of mitochondrial genome as well.

Aim

We aimed to investigate the entire mitochondrial genome in different adenoma types.

Material and methods

We collected 22 gonadotroph (GO), 11 GH producing (GH) and 11 null-cell (NC) adenoma specimens from samples removed by transsphenoidal surgery. From fresh frozen tissues DNA extraction was performed using QIAamp Fast DNA Tissue Kit. For library preparation VariantPro Amplicon Mitochondrion Panel kit was used. The total mtDNA (16569 bp) was sequenced on Illumina MiSeq Instrument. Following complex bioinformatic analysis Revised Cambridge Reference Sequence (rCRS) of the human mitochondrial DNA was used as reference. Heteroplasmy was determined using 3% cutoff.

Results

The whole mitochondrial genome were covered by 630 ± 370 (avg $\pm$ S.E.) reads per base. 496 variants were identified in adenomas compared to reference sequence. Overall a low (7.22%) heteroplasmy prevalence was found. Based on mitochondrial sequence variants by hierarchical cluster analysis we could not discriminate different adenoma types. No association between Ki-67 index or recurrent-nonrecurrent status of adenomas and mitochondrial variants were detected. Four variants appeared more often in null-cell adenomas compared to gonadotroph adenomas (chrM_188: 18% vs 0%, chrM_16093: 18% vs 0%, chrM_185: 27% vs 0% and chrM_14798: 36% vs 5%; Padj = 0.0246, 0.0246, 0.01542 and 0.01829, respectively). Of these variants chrM_14798, chrM_4216 and chrM_15452 are non-synonymous polymorphisms leading to amino acid change in MT-CYB (mitochondrially encoded cytochrome b) and in MT-ND1 (mitochondrially encoded NADH dehydrogenase 1) genes. We identified chrM_16189 variant (non-protein coding variant) in 40% (6/15) of nonrecurrent adenomas compared to recurrent ones where this variant was not present (0/11) ($P=0.0209$).

Conclusions

Next-generation sequencing is a reliable method for investigating mitochondrial genome and heteroplasmy in pituitary adenomas. In pituitary adenomas the prevalence of heteroplasmy of mitochondrial genome is low suggesting that these alterations may not influence mitochondrial function considerably. Of pituitary

tumours only null cell adenomas possess alterations of mitochondrial genome with potential functional consequences suggesting that during the development of this subtype of pituitary tumours mitochondrial function-associated mechanisms may have role.

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P780

Pituitary cell activation and recruitment in hipothyroidism

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Pituitary stem cells have been characterized in the postnatal pituitary. We now know they are organized in a niche and co-express specific markers such as Sox2, Sox9 or Gfra2. Although many studies by our group and others have been dedicated to its characterization *in situ* it is under discussion their role in the maintenance and turnover of the pituitary in physiological conditions or physiological pituitary challenges. It's not known if the stem cells are required and which molecular mechanisms are implicated in recruitment/differentiation. We established a model of hypothyroidism in rodents similar to human conditions in which levels of thyroxine are maintained just below the lower normal cut-off. We studied pituitary extracts in a precise time-course for stem cell and differentiation markers of thyrotropes. We have found that Shh is increased immediately after the establishing of the hypothyroidism. Following this, we purified the Gfra2+ stem cell population from vehicle and short-term hypothyroid animals and grown them as spheres in absence of serum. Spheres grow during the days of culture duplicating from day 1 to day 5 when they reach a plateau. Gfra2+ cells obtained from hypothyroid animals produce a significantly higher level of spheres per well both at day 1 and at day 5. When cultured in presence of cyclopamine, a Shh inhibitor, the number of spheres is significantly reduced in the hypothyroid Gfra2+ but not in the control wells. We used immunofluorescence techniques to see what happen in the intact pituitary niche in vivo. A genetic mouse model of tracing where Gfra2/Sox2 positive cells are induced to express GFP long-term after the tamoxifen injection was followed in a time-course under the same conditions of above vehicle/hypothyroidism. There was a significant increase of the Sox2 positive cell in long-term hypothyroid mice compared with vehicle treated. Tracing the GFP+ population through a time-course, we detected a significant increase in the double GFP/TSH+ cells in the adenopituitary of hypothyroid mice compared to vehicle treated. This data confirm that Sox2 positive cells recruited from the pituitary niche are able to differentiate into TSH producing cells. In summary, our results indicate that the Gfra2/Sox2 population, the pituitary stem cells, are activated when a mild hypothyroidism is induced. Results *in vitro* and *in vivo* confirm that initially (short-term hypothyroidism) the stem cells are driven to proliferate and expand while later (long-term hypothyroidism) differentiate into thyrotropes.

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P781

SOM230 exerts anti-proliferative actions by reducing phospho-ERK1/2 levels in ACTH-secreting pituitary tumour cells

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Currently, the multi-ligand somatostatin (SS) analogue pasireotide (SOM230) is the only pituitary-targeted drug used to treat patients with Cushing's disease. SOM230 displays the highest affinity to somatostatin receptor type 5 (SSTR5) and compared to octreotide resulted more effective in reducing ACTH release. Despite its anti-secretory role, SOM230 has been associated with tumor shrinkage in patients subjected to long term treatment, although to date the key factors involved are poor elucidated. The present work aimed to investigate the molecular mechanisms implicated in SOM230-induced cytostatic and cytotoxic effects in ACTH-secreting primary tumour cultures and murine corticotroph tumour cells line, AtT-20. First, by western blot we found SSTR5 expressed at comparable levels in 17 different ACTH-secreting pituitary samples, whereas SSTR2 was detectable in 15 out of 17 tissues. SSTR5 and SSTR2 were expressed in AtT-20 cells. Then, we tested the effect of 96h stimulation with 1 μM SOM230 on cell proliferation in 6 different ACTH-secreting tumors by measuring 5-bromo-20-deoxyuridine incorporation during DNA synthesis. We found a significant *in vitro* suppression of cell growth in half of the analyzed samples ($-12.1\pm4.3\%$, $P<0.01$). Accordingly, SOM230 significantly inhibited cell growth in a dose-dependent manner in AtT-20 cells ($-10.5\pm7.7\%$ at 10 nM, $P<0.05$; $-3.9\pm10.9\%$ at 100nM, $P<0.05$; $-26.8\pm8.9\%$ at 1 μM, $P<0.01$), whilst octreotide was effective only at 1 μM ($-13.3\pm9.1\%$, $P<0.05$). To investigate whether direct antiproliferative actions SOM230-mediated might involve MAPK and cyclins pathways, we evaluated the expression level of phospho-ERK1/2 and CD1 in ACTH-secreting primary cultures exposed to 1 μM of SOM230. SOM230 reduced phospho-ERK1/2 levels in 5 of 8 tumours tested ($-36.4\pm20.5\%$, $P<0.01$), whereas no significant difference was found in CD1 expression levels in 3 tumours. These data were further confirmed in AtT-20 cells, where octreotide did not have any effect. Furthermore, we found that 48h incubation with 1 μM SOM230 was able to induce a significant increase of caspase 3/7 activity in 2 of 4 ACTH-secreting primary cultures ($17\pm3.6\%$, $P<0.05$). Altogether these data suggest a downstream implication of phospho-ERK1/2 inhibition in ACTH-secreting pituitary tumour cells by SOM230 resulting in cell proliferation suppression and indicating that broader-spectrum SS analogues may play a crucial role in the treatment of tumours where the MAPK pathway is overactivated. Moreover, we describe a pro-apoptotic effect of SOM230. Ongoing experiments are aimed to discriminate the specificity effects played by SSTR5 and SSTR2.

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P782

Abstract withdrawn.

P783

Corticotroph pituitary adenomas: the functioning vs the silent: a gene expression study comparing differentially expressed genes in the regulation of POMC

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Background
The exact mechanism behind the hypersecretion of ACTH and lack of negative cortisol feedback on POMC regulation in functional corticotroph adenomas (FCA) is unknown. Silent corticotroph adenomas (SCA) express, but do not secrete functional ACTH and have lower POMC expression. Using RT-qPCR and immunohistochemistry, previous studies have identified some POMC-transcription factors, regulators and processing enzymes to be differentially expressed