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Background
Variants of the *TSHR* gene occur in different types of thyroid tumors. The importance of their molecular testing and the clinical impact is unclear. The aim of this study was to detect *TSHR* variants in a large cohort of malignant, low-risk and benign thyroid tumors and correlate them with clinical and histopathological data.
Methods
The cohort consisted of 885 fresh frozen thyroid tumor samples (455 malignant tumors, 52 low-risk neoplasms and 378 benign tumors) from 148 pediatric (under 18 years) and 736 adult patients. DNA extracted from the samples was analyzed for the presence of *TSHR* variants (NM_000369.5) in exon 10 using the Nextera XT DNA Library Prep Kit and next-generation sequencing (MiSeq, Illumina).
Results
A total of 14 types of *TSHR* variants were found in 38 thyroid tumors from 37 patients (32 females, 5 males). The histology of *TSHR* -positive thyroid tumors was as follows: 30/378 (7.9%) benign tumors, 4/390 (1.0%) papillary thyroid carcinomas (PTCs), 3/52 (5.8%) low-risk neoplasms and 1/20 (5.0%) follicular thyroid carcinomas (FTCs). One-third 13/37 (35.1%) of patients with *TSHR* -positive thyroid tumor were pediatric patients. In pediatric patients, almost all (11/13) *TSHR* -positive thyroid tumors were benign, 1/13 was a low-risk neoplasm, and 1/13 was a PTC that was positive for the *NCOA4/RET* fusion gene and the *TSHR* variant K340N was found to be germline. In other PTC from an adult patient, the *TSHR* I541V variant was also found to be of germline origin. In the remaining three *TSHR* -positive thyroid carcinomas, peripheral blood from patients was unavailable. Overall, the most common variant was the *TSHR* M453T detected in eight samples with different histology (5× benign tumor, 1× low-risk neoplasm, 1× PTC, 1× FTC). The second most common variants detected in five cases each were D633Y and F631L. Both variants were identified only in benign tumors and interestingly, the D633Y variant was found only in pediatric patients. Other *TSHR* variants that were repeatedly detected only in benign tumors were: S425I in four cases, D633H in three cases, and T632I, I568T, and D619G each in two cases.
Conclusion
TSHR variants were detected at a higher frequency in thyroid tumors from pediatric (8.8%) than from adult (3.3%) patients. Most *TSHR* -positive thyroid tumors were benign and some of the variants found were associated only with benign histology.
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PS2-11-09

Dual agonism of sodium iodide symporter function *in vivo*

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Objectives
New approaches are urgently needed to enhance radioiodide (RAI) ablation of aggressive and metastatic thyroid cancer. Our previous experiments revealed that valosin-containing protein inhibitors (VCPi), such as disulfiram, markedly increase sodium iodide symporter (NIS) activity to promote RAI uptake. Recently, disulfiram was reported to inhibit NPL4 activity – a critical VCP cofactor – via its copper bound diethyldithiocarbamate metabolite $Cu(DDC)_2$. We hence hypothesised that disulfiram and its metabolites increase RAI uptake by interfering with ER-Associated Degradation (ERAD) via a VCP/NPL4 pathway, permitting more NIS protein to be trafficked to the plasma membrane. Here, our aim was to understand the mechanistic impact of $Cu(DDC)_2$ on NIS function in thyroid cells, as well as to investigate the clinical relevance of $Cu(DDC)_2$-gene interactions.
Methods
We utilised RNA-Seq to identify transcriptional pathways altered by $Cu(DDC)_2$. Technetium-99m pertechnetate (^{99m}Tc) uptake after intravenous administration was used to evaluate NIS function in wild-type BALB/c mice. TCGA was appraised to investigate $Cu(DDC)_2$-gene interactions with recurrence in RAI-treated papillary thyroid cancer (PTC).
Results
$Cu(DDC)_2$ increased RAI uptake in a dose-dependent manner across multiple thyroid cancer cell lines (mean ~3.4-fold). Subsequent RNA-Seq analysis revealed potent transcriptional changes in 8505C cells treated with $Cu(DDC)_2$, including dysregulation of 357 genes encoding transcription factors. TaqMan RTPCR confirmed induction of transcription factors with key roles in regulating NIS expression, such as PAX8 and CREM, in multiple thyroid cell lines and human primary thyrocytes. In support, $Cu(DDC)_2$ was unable to induce NIS mRNA expression or ^{125}I uptake when PAX8 was depleted in primary thyrocytes and thyroid cancer cells. Importantly, significant induction of thyroidal ^{99m}Tc-uptake (~30%;$n = 7$;5 mg/kg dose;$P < 0.05$) in wild-type BALB/c mice treated intravenously with $Cu(DDC)_2$ was associated with increasing PAX8 (1.4-fold;$P < 0.05$) and CREM mRNA (1.6-fold;$P < 0.01$) expression. Surprisingly, $Cu(DDC)_2$ retained activity in the absence of NPL4 but not VCP in thyroid cancer cells and primary thyrocytes. Thus, $Cu(DDC)_2$ required functional VCP but not NPL4 expression to enhance RAI uptake. We further appraised TCGA with LASSO regression analysis identifying a 22-gene riskscore classifier based on $Cu(DDC)_2$-associated transcription factors, which showed a significantly worse prognosis for high-risk RAI-treated PTC [Hazard Ratio (HR)=11.6;95%CI 5.8-23.31;$P < 0.001$;$n = 256$].
Conclusions
We have identified a new dual agonist of RAI uptake dependent on the distinct functionalities of PAX8 and VCP, with the potential to directly impact RAI therapy for patients with aggressive thyroid cancer. Our bioinformatic analyses validated the clinical relevance of $Cu(DDC)_2$-associated genes in RAI-treated PTC, enabling construction of a risk score classifier for predicting recurrence.
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PS2-11-10

Exploring the genetic links between voltage-gated potassium channels and familial non-medullary thyroid carcinoma: a family study

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Introduction
Our team identified a family where 5 elements developed thyroid cancer between the ages of 26 and 38. Since no syndromic form of the disease was found, the diagnosis was of familial non-medullary thyroid carcinoma (FNMTC). Our team employed Whole-Exome Sequencing (WES) and identified a new potentially pathogenic germline mutation in the *KCNB2* gene [*p.(Gly106Arg)*]. *KCNB2* encodes a voltage-gated potassium channel (vgKCN), and the detected missense mutation is localized in the tetramerization domain of the protein, possibly affecting its assembly and K^+ efflux. Since K^+ efflux by the cell is a necessary condition for cellular homeostasis, channel disruption can impact the function of other ion channels nearby. Mice studies showed that *KCNE2* disruption indirectly impairs sodium-iodide symporter (NIS) function, and therefore iodide uptake by the cell, resulting in hypothyroidism or follicular nodular disease.
Hypothesis
By indirect effect on NIS function vgKCN mutations may increase predisposition to thyroid cancer and interfere with radioiodine (RAI) therapy response.
Methodology
We conducted *in silico* studies using two different NGS databases, TCGA and one in-house oncocytic tumors database (513 and 18 patients, respectively). Alterations in 59 genes were searched for copy-number variation, point mutations and other genetic alterations. *in vitro* assays using the FRTL-5 cell line are being performed. FRTL-5 cells were transfected with overexpression vectors containing either *KCNB2* wild-type or *KCNB2* mutated sequences, and the empty vector (EV) as a negative control. Expression of thyroid markers (e.g. NIS, TSH receptor, Thyroglobulin and TPO) was evaluated by qPCR and cell viability by PrestoBlue assay. Protein expression of thyroid markers will be assessed by Western blot. Cell cycle and apoptosis through flow cytometry, cell morphology by phalloidin assay, and cell colony formation by crystal violet. Transformed cells will further be treated with Guangxitoxin-1E, a potent *KCNB1* / *KCNB2* inhibitor.
Results
Our *in silico* results show that vgKCN mutations are rare events in thyroid cancer [19/488 (4%) in TCGA; 3/18 (17%) in our in-house database]. *BRAF* and *NRAS* alterations are frequent events in vgKCN altered tumors (58% and 16%, respectively). No *KCNB2* pathogenic mutations were observed. vgKCN mutations were not correlated with patient prognosis. Our *in vitro* preliminary results show that *KCNB2* mutated cells present higher expression of the channel than *KCNB2 wild-type* cells. No differences in cell viability were found between *KCNB2* wild-type and mutated cells.
Conclusions
If confirmed, vgKCN mutations may identify patients with altered RAI response, serving as thyroid cancer markers and potential pharmacological targets.

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Diagnosis of Thyroid Cancer-1

PS-12-01

The clinical significance of markedly elevated preoperative serum thyroglobulin levels

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Objectives
Any condition involving increased follicular cell mass may raise serum thyroglobulin levels. However, we occasionally encounter patients with markedly elevated preoperative thyroglobulin levels, indicating thyroid cancer with substantial metastatic burden. We aimed to investigate the clinical significance of markedly elevated preoperative serum thyroglobulin levels.
Methods
From 2019 to 2021, we analyzed patients underwent thyroid surgery for papillary thyroid cancer (PTC) or benign, with a focus on preoperative thyroglobulin levels $\geq$500ng/ml.
Results
In 7,737 PTC cases and 781 benign cases, 84 in each group had preoperative thyroglobulin levels $\geq$500ng/ml. Forty (48%) had the BRAF V600E mutation, and 11 (13%) had the TERT promoter mutation in PTC. In the 24 cases with a cancer size $>$4 cm, 3 (13%) showed distant metastasis, and 1 (4%) had another nodule $>$2 cm; none had diffuse thyroid disease (DTD). In the 32 cases with a cancer size $>$2 cm but $\leq$4 cm, 4 (13%) showed distant metastasis. 4 (12%) had another nodule $>$2 cm, and 3 (9%) exhibited DTD; two had both conditions. In the 28 cases with a cancer size $\leq$2 cm, 1 (4%) showed distant metastasis. 15 (53%) had another nodule $>$2 cm, and 9 (32%) exhibited DTD; two had both conditions. In benign, for 50 cases with size $>$4 cm, multinodular, 1 (2%) exhibited DTD; for 12 cases with size $>$2 cm but $\leq$4 cm, 3 (25%) cases showed DTD; and for 22 cases with size $\leq$2 cm, no focal lesion, 22 (100%) cases exhibited DTD. Even with preoperative thyroglobulin $\geq$1000ng/ml, a similar pattern persisted.
Conclusions
In cases with markedly elevated preoperative thyroglobulin levels, a high cancer burden is often observed. However, it can also occur in cases with large benign nodules or DTD. Therefore, additional research is needed to determine the clinical utility when excluding such cases.

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PS2-12-02

Performance of eu-tirads, ATA and AACE/ACE-AME ultrasound risk stratification system (RSS) in pediatric patients with thyroid nodules

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Objectives
Thyroid nodules in patients $\leq$18 years are less frequent than in adults, however several studies showed a higher rate of malignancy. The aim of the present study is to analyze the ultrasound (US) features of nodules in patients $\leq$18 years and to test the ability of the main US risk stratification system (RSS) in identifying malignancy. Moreover, we also evaluated the potential correlation between the results of US RSS and cytology.
Methods
We analyzed the US reports and cytology results of series of a consecutive thyroid nodules in patients $\leq$18 years diagnosed in our department for the first time, between 2016 and 2022. The nodules were classified according to EU-TIRADS, 2015-ATA, and AACE/ACE-AME while the cytology was classified according to Italian Consensus.
Results
The whole study group consisted of 271 nodules in 221 patients. Most patients were females (74.2%), and the median age at cytology was 16 years (IQR 14-17). The median nodule diameter was 1.9 cm (IQR 1.4-2.9). Cytology result was TIR5 in 9.3%, TIR4 in 4%, TIR3b in 8.8%, TIR3a in 19.5%, TIR2 in 32.7%, TIR1C in 13.6% and TIR1 in 12.1% of cases. Ultrasound features were available in 216/271 nodules (79.7%). The thyroid was multinodular in 34.3% of cases. The nodules were mostly solid (74.1%), isoechoic (49.1%), “wider than tall” (80.6%) with well-defined margins (82.9%) and without calcifications (77.8%). Approximately one third of the nodules had a high suspicion of malignancy in accordance with the 3 RSS evaluated [EU-TIRADS 5: 35.2%; 2015-ATA High Suspicion 30.6%; AACE/ACE-AME High-risk: 35.2%]. The rate of cytology suspicious for malignancy (TIR4 and TIR5) was 23.7-27.3% in the high and 10.5-11.4% in the low/intermediate ultrasound risk.
Conclusions
Our data show a lower performance of US RSS in thyroid nodules for pediatric patients compared to adults. However, in patients $\leq$18 years, the 3 main US RSS were able to identify about 75% of the nodules as low/intermediate suspicious for malignancy of which about 90% were not suspicious for malignancy by cytology.