Evidence for a role of the amyloid precursor protein in thyroid carcinogenesis

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Abstract

We have recently found an increased expression of amyloid precursor protein (APP) in cold thyroid nodules that are difficult to classify as a truly benign thyroid neoplasm or a lesion with the potential for further dedifferentiation. Since differences in APP activity have been found in other human cancers, we asked whether thyroid carcinogenesis might be associated with an altered APP expression and function. APP regulation was studied in vitro in differentiated (FRTL-5) and dedifferentiated follicular thyroid carcinomas (FTC-133) thyroid cells after specific inhibition or activation of the cAMP-PKA, the PI3K/AKT or the protein kinase c (PKC) cascades. In vivo analysis of APP expression and downstream signalling was performed in benign and malignant thyroid tissues. We found that upregulation of APP expression and sAPP secretion is induced by TSH in differentiated thyroid cells and by insulin in thyroid cancer cells. PKC is a strong activator of APP cleavage and in FTC-133 confers prolonged release of the APP ectodomain. FTC-133 but not FRTL-5 cells show a prominent cell surface expression of the APP ectodomain, which has been suggested to function as an autocrine growth factor. Thyroid cancers are characterized by APP upregulation, increased membrane targeting of the APP ectodomain and significantly increased mRNA levels of the APP scaffold proteins JIP1, ShcA and Fe65.


Introduction

Aberrant processing of the amyloid precursor protein (APP) and its formation in plaques play a major role in Alzheimer's disease pathogenesis. Besides the central nervous system, APP is also highly expressed in the epidermis, the pancreas and the thyroid (Beyreuther & Masters 1997, Pietrzik et al. 1998, Hansel et al. 2003). APP is a transmembrane protein and contains a large N-terminal ectodomain and a short C-terminal endodomain (APP C-terminal intracellular domain (AICD); Kang et al. 1987). APP is sequentially cleaved by different secretases. The major proteolytic pathway is the non-amyloidogenic secretory pathway, where an α-secretase cleaves the ectodomain, followed by the secretion of sAPP, the soluble ectodomain (De Strooper & Annaert 2000). The C-terminal membrane-bound fragment is further cleaved by γ-secretases generating the intracellular C-terminal domain, termed AICD.

Recent data propose that APP plays a role in cell adhesion, motility, proliferation (Kummer et al. 2002, Siemes et al. 2004) as well as in neuroprotection and neurite outgrowth (Salinero et al. 2000, Masters & Beyreuther 2006) and in vivo data suggest that sAPP functions as an autocrine growth factor (Pietrzik et al. 1998, Schmitz et al. 2001, Siemes et al. 2004). Multiple lines of evidence show that the shedding of APP by the α-secretase is regulated by the protein kinase C in non-neuronal systems and brain (Buxbaum et al. 1993, Hung et al. 1993, Mills & Reiner 1999). Additionally, with its targeting to the cell membrane and since it structurally resembles a receptor, APP has also been suggested to play a role in signal transduction and gene transcription (Rossjohn et al. 1999, Hebert et al. 2006). The AICD contains a conserved YENPTY motif that enables interaction with a number of binding proteins via their phosphotyrosine-binding domain, for example with Fe65, JNK interacting protein (JIP1) and ShC proteins (De Strooper & Annaert 2000). In particular, the APP adaptor protein Fe65 is involved in nuclear signalling of APP and in cell damage response (von Rotz et al. 2004, Yang et al. 2006). In addition, APP has been linked to the pro-apoptotic JNK pathway via JIP1 (Matsuda et al. 2001, Taru et al. 2002). Docking of the APP PTY domain to Shc protein families, for example, ShCA, promotes the APP mediated signal transduction via the MAPK and PI3K/Akt pathways (Liao et al. 2004, Lichtenthaler 2006).
Further studies provide first evidence that APP may be associated with tumorigenesis. For example, in oral squamous cells, the mRNA expression of APP was significantly higher in cancerous tissue compared with normal tissue. In addition, a higher expression of several interactor proteins of AICD was found in pancreatic carcinoma cells (Hansel et al. 2003).

The role of APP in the human thyroid has not been fully elucidated. Previous studies describe APP as a potent stimulator of thyrocyte proliferation (Popp et al. 1996, Pietrzik et al. 1998). However, there are no data on a potential role of APP in thyroid tumorigenesis.

In a recent proteomic study, we have identified an increased expression of APP, the amyloid precursor-binding protein 1 (APPBP1) and the APP-p-component in benign cold thyroid nodules (CTNs) compared with normal thyroid tissue (Krause et al. 2007b). The molecular aetiology of CTNs is unknown and at present it is unclear, if the CTN represents a truly benign thyroid neoplasm or a lesion with the potential for further dedifferentiation. Since differences in APP activity have been found in other human cancers, we asked whether thyroid tumorigenesis and carcinogenesis may be associated with increased APP expression and function and how signalling pathways linked to thyroid proliferation, differentiation and dedifferentiation may affect APP expression.

### Materials and Methods

#### Tissue specimen

Thyroid samples were obtained from patients undergoing thyroid surgery for nodular thyroid disease or thyroid cancer. None of the patients received medical treatment for thyroid disease. Samples were obtained at surgery and were snap-frozen in liquid nitrogen for mRNA expression analysis. Duplicates of 22 benign thyroid tumours (BN; 12 follicular adenomas and 10 colloid nodules), 22 corresponding normal thyroid tissues (NTs), 14 follicular thyroid carcinomas (FTCs), 10 classic papillary thyroid carcinomas (PTCs) and 5 anaplastic thyroid carcinomas (ATCs) were studied for mRNA expression of APP interactor proteins. For immunohistochemical analysis of APP expression, paraffin-embedded tissue sections from 51 BNs (all follicular adenoma), 22 NTs, 56 FTCs, 11 PTCs and 2 ATCs were used. In addition, a Tissue Microarray (Biomol; Hamburg, Germany) comprising 11 PTCs, 5 FTCs, 2 ATCs, 5 BNs and 2 NTs (duplicates of each specimen) was used. There was an overlap of 22 BNs and the corresponding normal tissue and 14 FTCs that were analysed by quantitative RT-PCR and immunohistochemistry.

Thyroid samples were obtained from the Department of Surgery, University of Halle, and the Departments of Pathology from the universities of Leipzig and Essen as well as from local Leipzig hospitals. Informed consent was obtained from all patients and the local ethics committee approved the study.

![Figure 1](https://example.com/figure1.png) **Figure 1** APP processing is regulated by TSH and insulin. Western blots showing effects of TSH and insulin stimulation on sAPP secretion and APP expression. (A) FRTL-5 cells or (B) FTC-133 cells were stimulated with 10 mU/ml TSH or 100 ng/ml insulin for 4 and 24 h. Controls contained DMSO as vehicle only. Representative blot of three independent experiments is shown using antibodies against the APP ectodomain and endodomain as described in Materials and Methods.

![Figure 2](https://example.com/figure2.png) **Figure 2** sAPP release is regulated by protein kinase C. Western blots showing the sAPP secretion after stimulation of FRTL-5 cells and FTC-133 cells with either the PKC agonists PMA (1 μM), or the antagonist GF109203X (5 μM), or treatment with both 1 μM PMA + 5 μM GF109203X. Treatment with PMA led to a raise in sAPP release whereby treatment with GF109203X had no effect. Co-treatment with both, PKC agonist and antagonist completely blocked sAPP secretion. PKC activation enhances sAPP secretion without changing the amount of holo APP. Shown is a representative blot of three independent experiments using an antibody against the APP ectodomain as described in Materials and Methods.
Cell culture

FRTL-5 rats and FTC-133 cells (human FTC-cell line) were split into six-well plates (2.5 × 10⁵/well for FRTL-5 and 0.5 × 10⁵/well for FTC-133). FRTL-5 cells were cultured in a 2:1:1 mixture of Dulbecco’s modified Eagle’s medium (DMEM):Ham’s F12:MCDB104 (all from Invitrogen) supplemented with 5% calf serum and 1% penicillin-streptomycin (both from Gibco Life Technologies), 10 μg/ml insulin, 0.4 μg/ml hydrocortisone, 45 μg/ml ascorbic acid (all from Sigma–Aldrich), 5 μg/ml transferrin (Calbiochem, Darmstadt, Germany) and 5 mU/ml bovine thyrotrophin (TSH) (Sigma–Aldrich). FTC-133 cells were maintained in DMEM containing 10% calf serum and 1% penicillin-streptomycin (Invitrogen). Prior to TSH, forskolin or insulin stimulation, cells were starved for 72 h in medium containing 0.2% serum without TSH or insulin, respectively. Afterwards, cells were stimulated with 10 mU/ml TSH, 5 mM forskolin (Sigma–Aldrich) or 100 ng/ml insulin for 24 h.

For stimulation or inhibition of protein kinase C, cells were either treated with 1 μM phorbol 12-myristate 13-acetate (PMA) or 1 μM phorbol 12-myristate 13-acetate (PMA).

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![Figure 3](image-url) Protein kinase C-stimulated sAPP release is prolonged in thyroid carcinoma cells. FRTL-5 cells and FTC-133 cells were stimulated with 10 nM, 100 nM and 1 μM PMA which led to a dose-dependent increase in sAPP secretion in both cell lines after 4 h. However, in contrast to FRTL-5 cells, the PKC-stimulated sAPP release is prolonged over 24 h in FTC-133 cells. Shown is a representative blot of three independent experiments using antibodies against the APP ectodomain and endodomain as described in Materials and Methods.

![Figure 4](image-url) In vitro cell surface targeting of the APP ectodomain is increased in malignant thyrocytes. In order to assess different subcellular localizations of the APP endodomain and ectodomain, FRTL-5 cells and FTC-cells were dually labelled with an antibody against the endodomain (green) and against the ectodomain (red). Overlays show the increased cell surface targeting of the APP ectodomain in FTC-133 cells compared with a predominantly cytoplasmatic expression in FRTL-5 cells.
(PMA) or with 5 μM bisindolylmaleimide (GF10923X; all from Calbiochem) for different periods of time. Control cells were treated with the vehicle dimethyl sulphoxide (DMSO) only. After stimulation, supernatants were collected for sAPP detection and PBS washed cells were shock-frozen in liquid nitrogen and stored at –80 °C.

**Western blot analysis**

For the detection of APP protein expression, cells were lysed in buffer containing 10 mM Tris, 400 mM NaCl, 1 mM EDTA, 0.1% NP-40 (all from Sigma–Aldrich). For the detection of sAPP, supernatants were centrifuged using Centricon Centrifugal Filter Units YM-10 (Millipore,
Figure 5. Altered expression of the APP endodomain and localization of the APP ectodomain in thyroid carcinomas.

Representative images of the localization of (A) APP endodomain and (B) APP ectodomain in three benign and malignant thyroid tissue samples: (a) normal thyroid tissue, (b) benign thyroid tumours, (c) follicular carcinoma, (d) papillary carcinoma and (e) anaplastic carcinoma. Semiquantitative analysis was performed by calculating the percentage of positively stained thyrocytes/six randomly selected sections/slide (×200 magnification, duplicates). The degree of staining was quantified as follows: −, no positive staining; +, <30% positive staining; ++, 30–60% positive staining and ++++, >60% staining. (f) The histograms summarize the results of the semiquantitative analyses. (G) Distinct cell surface expression of the APP ectodomain: in follicular adenoma, follicular carcinoma and papillary carcinoma, the APP ectodomain is found to be clustered and to form patches at the cell surface (arrows) whereas in normal tissue the APP ectodomain is prominently expressed in the cytosol (×1000 magnification).

Bedford, MA, USA). After protein quantification using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA), 30 μg protein were separated on 8 and 12% SDS gels for the detection of sAPP and APP respectively. After semi-dry western blotting, membranes were blocked with 5% BSA in TBST (50 mM Tris–HCl (pH 7.5) and 150 mM NaCl containing 0.05% Tween-20). Blots were probed overnight with the following primary antibodies: anti-APP C-terminus/endodomain (Sigma–Aldrich) and anti-APP N-terminus/ectodomain (22C11; Chemicon; Temekula, CA, USA) diluted (1:1000 for APP anti C-terminus and 1:5000 for 22C11) in TBST (10 mM Tris pH 8.0, 150 mM NaCl, 0.05% Tween-20) containing 5% BSA. Blots were reprobed with secondary antibodies coupled to horseradish peroxidase at a dilution of 1:10 000 (Cell Signaling; Charlottesville, VA, USA) and visualized by enhanced chemiluminescence (Pierce).

Confocal laser scanning microscopy

FRTC-133 and FRTL-5 cells were plated on coverslips (0.5×10⁵/coverslip for FRTC and 2.5×10⁵/coverslip for FRTL-5) and cultured as described above. After 2 days, cells were fixed and permeabilized with 0.05% Triton X-100 in 2% paraformaldehyde/PBS (all from Sigma–Aldrich) for 30 min on ice. Afterwards, cells were washed three times with PBS followed by incubation for 1 h on ice with 22C11 for the APP N-terminus and a C-terminal APP antibody (1:2000 dilution in PBS). Subsequently, cells were washed three times with PBS and were incubated for 1 h on ice with the secondary anti-rabbit Alexa–Fluor 488 antibody (for C-terminus; 1:1000 in PBS) or anti-mouse Alexa–Fluor 546 antibody (for N-terminus; 1:1000 in PBS). Secondary antibodies were obtained from Molecular Probes (Eugene, OR, USA). Coverslips were mounted in Entellan (Merck).

Confocal analysis was performed on a confocal laser scanning system (TCS SP2, Leica; Wetzlar, Germany) attached to a microscope (DM IRBE, Leica) with ×100 oil immersion lens (PL Fluotar 1.3). Optical sections (0.45 μm) with ~150 cells were investigated.

Immunohistochemistry

Paraffin-embedded sections (2 μm) were deparaffinized in xylol and subsequently pretreated in a microwave oven in 0.1 M citrate buffer (pH 6) to allow antigen retrieval. The LSAB+ System (DAKO Cytomation; Hamburg, Germany) was used for immunodetection according to the manufacturers’ description. Briefly, slides were incubated with 3% H₂O₂ for 30 min followed by three washing steps with PBS including 1% BSA (PBS/BSA; Sigma). Subsequently, unspecific binding was blocked for 30 min followed by treatment with specific APP antibodies (1:500 for C-terminal antibody (Sigma–Aldrich) and 1:25 for N-terminal antibody (Chemicon)). After incubation with a biotinated secondary anti-rabbit antibody, the immunocomplexes were detected by streptavidinperoxidase and diaminobenzidin. The sections were counterstained with hemalaun and mounted in Aquatex (Merck). Semiquantitative analysis of APP staining was done by two independent investigators (S K/D F) by determining the percentage of positively stained thyrocytes/six randomly selected sections/slide (×200 magnification) and quantified as follows: −, no staining; +, <30% staining; ++, 30–60% staining and ++++, >60% staining. (Krause et al. 2007a).

RNA extraction and RT-PCR

RNA was isolated from snap-frozen thyroid tissue using Trizol reagent (Invitrogen). One microgram RNA per sample was reverse transcribed in a final mixture of 5 × First-Strand Buffer (250 mM Tris–HCl (pH 8.3), 375 mM KCl, 15 mM MgCl₂), 0.5 mM dNTPs, 5 mM dithiothreitol, 15 U Prime RNase Inhibitor (PeqLab, Erlangen, Germany), 0.5 μg random hexamer primers and 200 U Moloney murine leukaemia virus reverse transcriptase (GibcoBRL). Reverse transcription was performed at 37 °C for 60 min and 94 °C for 5 min. Consistent expression of house-keeping genes (β-actin and GAPDH) was demonstrated in all samples by RT-PCR as described previously (Krause et al. 2007a).
Real-time PCR

To quantify changes in mRNA expression patterns of Fe65, ShcA and JIP1, real-time PCR was performed using the LightCycler (Roche) and intron-spanning primers for JIP1, ShcA and Fe65 as described elsewhere (Hansel et al. 2003). mRNA expression was normalized for β-actin mRNA levels (Krause et al. 2007a). RT-PCR conditions were as follows: after initial denaturation (30 s) at 95 °C, RT-PCR was carried out for 40 cycles at 56 °C for 7 s and 72 °C for 7 s, 4 mM MgCl₂ for JIP1; 57 °C for 7 s and 72 °C for 4 s, 3 mM MgCl₂ for ShcA; 59 °C for 7 s and 72 °C for 9 s, 3 mM MgCl₂ for Fe65.

Fold upregulation or downregulation of mRNA expression was calculated as follows:

\[
\text{Fold change} = 2^{\frac{\text{threshold cycle of gene NT} - \text{DT}}{\text{threshold cycle \( \beta \)-actin NT} - \text{DT}}}
\]

‘Diseased tissue’ (DT) corresponds to BN, FTC, PTC and ATC, whereas ‘normal tissue’ (NT) corresponds to the surrounding tissue of benign thyroid nodules (BNs). The Mann–Whitney U test was applied to calculate the statistical significance of differences in APP mRNA expression between thyroid tissues.

Results

Influence of different signalling cascades on APP expression and processing in thyrocytes

In the thyroid, the regulation of proliferation and differentiation depends on three signalling pathways. The TSH-cAMP-protein kinase A cascade stimulates thyroid function and induces differentiated cell proliferation under a co-mitogenic (depending on the thyroid cell system) influence of the insulin/PI3K signalling pathway (Kimura et al. 2001). Activation of protein kinase C also induces cell proliferation, but this is accompanied by dedifferentiation (Roger & Dumont 1984, Medina & Santisteban 2000, Kimura et al. 2001).

To investigate how these different pathways linked to thyroid proliferation, differentiation or dedifferentiation affect APP expression, we used a differentiated thyroid cell line (FRTL-5) and a thyroid carcinoma cell line (FTC-133). In the first set of experiments, FRTL-5 and FTC-133 cells were incubated with 10 mU/ml TSH or 100 ng/ml insulin for different periods of time. As demonstrated in Fig. 1, in FRTL-5 cells treated with

Figure 6 mRNA expression of the APP scaffold proteins is increased in thyroid carcinomas compared with benign thyroid tissue. Increased expression of (A) Fe65, (B) ShcA and (C) JIP1 in thyroid carcinomas compared with benign thyroid tumours and normal thyroid tissue. mRNA expression of each gene was normalized for β-actin expression. BN, benign thyroid tumours; NT, normal tissue; FTC, follicular thyroid carcinoma; PTC, papillary thyroid carcinoma and ATC, anaplastic thyroid carcinoma. Box plots show median and distribution (box area = 50% of samples) of Fe65, ShcA and JIP1 in the thyroid tissues normalized for β-actin mRNA expression. *represent outliers.
TSH, the APP expression increases after 24 h whereas the sAPP secretion increased time dependently compared with the control (Fig. 1A). Similarly, stimulation with insulin induced the secretion of sAPP time dependently. After 24-h incubation with insulin, the sAPP secretion was three times higher than the control ($P<0.001$; Fig. 1A). In addition, insulin rapidly stimulates APP expression with a maximum of APP expression after 30 min. This suggests that insulin activates predominantly the α-secretory pathway (Fig. 1A).

In FTC-133 cells, TSH does not affect APP expression nor sAPP secretion (Fig. 1B) in line with lack of the endogenous TSH receptor in these cells. By contrast, incubation with 100 ng insulin time dependently stimulated sAPP secretion and furthermore APP expression with a maximum after 24 h (Fig. 1B).

To study PKC effects on APP processing, we incubated FRTL-5 and FTC-133 cells with the PKC activator PMA and the PKC inhibitor GF109203X. As shown in Fig. 2, incubation with 1 μM PMA for 24 h resulted in a significantly increased sAPP secretion ($P<0.001$) while co-treatment with 1 μM PMA and 5 μM GF109203X abolished this effect (Fig. 2). However, the enhanced sAPP secretion after PKC stimulation is not accompanied by an altered expression of holo APP (Fig. 2).

It has been suggested before that the protein kinase C family is implicated in thyroid carcinogenesis (Prevostel et al. 1995, Knauf et al. 1999, 2002). In view of the strong induction of sAPP release after PKC stimulation, we were interested if the kinetics of PKC mediated sAPP release differs between benign and malignant thyroid cells. Application of increasing concentrations of PMA (10 nM, 100 nM and 1 μM) dose dependently enhanced the release of sAPP in both FRTL-5 and FTC-133 cells after 4 h (Fig. 3). However, PKC activation in FTC-133 cells resulted in prolonged sAPP secretion over 24 h, whereas in FRTL-5 cells, the effect waned and sAPP secretion returned to basal levels (Fig. 3).

**Cell surface expression of the APP ectodomain is increased in malignant thyrocytes in vitro**

APP function results from the secretion of the mitogenic ectodomain or from the endodomain linking APP to intracellular signalling via scaffold proteins. This prompted us to investigate the cellular localization of the APP ectodomain and endodomain in differentiated thyroid cells and in thyroid carcinoma cells. To this aim, confocal microscopy of FRTL-5 and FTC-133 cells was performed with dually labelled monoclonal antibodies against the APP endodomain and ectodomain.

Using the antibody directed against the APP endodomain, we found a diffuse intracellular APP distribution with a perinuclear accumulation in differentiated and in thyroid carcinoma cell lines. By contrast, striking differences were observed in the localization of the APP ectodomain. FTC-133 cells showed increased cell surface targeting, whereas a cytosolic localization was found in FRTL-5 cells (Fig. 4).

**Upregulation of APP protein expression and increased cell membrane targeting of the APP ectodomain are features of thyroid cancers in vivo**

In view of the finding of a distinct localization of the APP ectodomain in FTC-133 cells versus FRTL-5 cells, we hypothesized that this difference may also exist in thyroid malignancies. Thus, we performed immunohistochemistry on a panel of BNs, normal thyroid tissues (NTs) and thyroid cancers (FTC, PTC and ATC) using specific antibodies against the APP endodomain and ectodomain.

Similar to the in vitro situation in thyroid cell lines, we found that the APP endodomain is predominantly expressed in the cytoplasm of the thyrocyte with a significant upregulation in thyroid cancers compared with BNs and NT (Fig. 5A(f)). Moreover, thyroid carcinomas displayed increased expression of the APP ectodomain (PTC>FTC>ATC) compared with benign thyroid nodules (BNs) and normal thyroid tissue. Regarding the localization (similar to the in vitro situation in FTC-133 cells) a strong punctuated basolateral membrane staining was found exclusively in thyroid carcinomas and to a lesser degree also in follicular adenoma, whereas in NT (similar to the in vitro situation in FRTL-5 cells), a predominantly intracellular staining was observed (Fig. 5C).

It has been suggested that the growth-promoting effects of sAPP, the secreted APP ectodomain, are mediated via a cell surface receptor (Mattson 1997). This hypothesis could explain the particular punctuated accumulation of APP ectodomain on the cell surface in thyroid carcinoma cells in vitro and in vivo.

**Downstream APP targets are upregulated in thyroid carcinomas**

Successive proteolytic processing of APP leads to the release of the APP C-terminal intracellular domain (AICD) that has been proposed to alter gene expression and apoptosis by interaction with scaffold proteins (De Strooper & Annaert 2000, Hansel et al. 2003). In view of the higher APP C-terminal protein expression in thyroid carcinomas, we then asked if this might have functional consequences and could result in increased APP mediated signalling. As an indirect measurement of AICD activity, we analysed the mRNA expression of three important APP interactors, Fe65, JIP1 and ShCA by quantitative real-time RT-PCR.

As shown in Fig. 6, the mRNA expression of Fe65, JIP1 and ShCA is significantly upregulated in thyroid carcinomas ($P<0.001$ for all three genes) compared with BNs and NTs.

**Discussion**

Thyroid tumours are a frequent finding in inhabitants living in iodine-deficient regions and a considerable portion of these tumours are CTNs. The molecular pathogenesis of benign...
CTNs is unknown. We recently performed a proteomic study of benign CTNs, where we found evidence for increased expression of APP, APP-binding protein and APP-p3-component in CTNs (Krause et al. 2007b). Since CTNs exhibit certain signs of dedifferentiation (e.g. loss of iodine uptake) and overexpression of APP was observed in other human cancers and has been linked to aberrant mitogenic signalling, we hypothesized that thyroid cell dedifferentiation and transformation might be linked with altered APP expression. Using immunohistochemistry, we found that dedifferentiation goes along with an increased APP expression. Thus, the highest APP expression levels were observed in thyroid cancers compared with BNs and normal thyroid tissues. Moreover, we found a strong punctuated cell surface expression of the APP ectodomain in thyroid cancers and to a lesser extent in follicular adenomas, which was not detected in normal thyroid tissue. Similar to the in vivo situation, an increased cell surface expression of the APP ectodomain was detected in FTC-133 but not in FRTL-5 cells (Fig. 4). It has been suggested that the growth-promoting effects of sAPP, the secreted APP ectodomain, are mediated via a cell surface receptor (Mattson 1997). This hypothesis could explain the particular punctuated accumulation of APP ectodomain on the cell surface in thyroid carcinoma cells in vitro and in vivo.

It is currently not known, how the different molecular pathways regulating thyroid proliferation, differentiation (PKA/PI3K cascade) and dedifferentiation (PKC cascade) affect APP expression and sAPP secretion. We therefore studied APP regulation in differentiated thyroid cells (FRTL-5 cells) and thyroid cancer cells (FTC-133 cells) using pharmacological activators of the respective signalling cascades. In FRTL-5 cells, we found a dependence of the APP expression and sAPP secretion on TSH, an observation which has been described before (Pietrzik et al. 1998). Moreover, in FRTL-5, cells insulin stimulates sAPP release without altering the APP expression. By contrast, in FTC-133 cells, only insulin induces APP expression and sAPP release. This result can be explained by the lack of endogenous expression of the thyroid-stimulating receptor in this cell line (Ros et al. 1999). Using forskolin as an endogenous activator of cAMP-PKA signalling, we were able to mimic the effects of TSH on APP processing in FRTL-5 cells (data not shown). Interestingly, PKC activation by PMA resulted in the strongest induction of sAPP secretion in both cell lines. Thereby, activation of PKC by PMA dose dependently leads to an enhanced sAPP secretion in both FRTL-5 and FTC-133 cells. However, this process was prolonged in FTC-133 cells, in which sAPP was accumulating even after 24-h incubation with PMA. By contrast, FRTL-5 cells displayed a peak in sAPP secretion after 4-h PMA stimulation, which then turned to basal levels. This is particularly interesting since the PKC family has been implicated in thyroid tumourigenesis (Dumont et al. 1992, Prevostel et al. 1995, Knauf et al. 2002, Eszlinger et al. 2005) and may imply a novel target of PKC activation in thyroid carcinomas.

Besides its role in proliferation, recent data suggest that APP acts at the cell surface to bind and activate signalling proteins via its intracellular domain (AICD), which is released after further cleavage of the APP endodomain by γ-secretases (De Strooper & Annaert 2000). Thus, the finding of the increased expression of the APP endodomain in thyroid cancers may propose a distinct APP mediated signal transduction. Indeed, we found a significantly increased mRNA expression of several AICD-binding proteins (Fe65, ShcA and JIP1) in thyroid malignancies compared with BNs and normal thyroid tissue.

JIP1 and Shc are relevant signalling proteins of the JNK pathway (JIP1) and the RAS/MAPK and the PI3K/akt (Shc) cascades (Pellicci et al. 1992, Matsuda et al. 2001, Taru et al. 2002a, King & Scott 2004). Fe65 has been shown to stimulate APP trafficking and APP proteolysis, which in turn leads to increased sAPP secretion (King & Scott 2004). A higher expression of Fe65 in thyroid carcinomas could enhance the secretion of sAPP, thereby contributing to cancer cell growth (Pietrzik et al. 1998).

In summary, our study provides evidence for a role of the APP in thyroid dedifferentiation and carcinogenesis. To follow-up on this hypothesis, further studies regarding APP function, for example, using knockout models are mandatory. In addition, further studies will be needed to fully elucidate the link between PKC activation, increased APP processing and thyroid dedifferentiation.

Declaration of Interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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