

Review Article

mRNA and microRNA expression patterns in adrenocortical cancer

Adrienn Zsippai, Diana Rita Szabó, Peter M. Szabó, Zsófia Tömböl, Melinda R. Bendes, Zoltán Nagy, Károly Rácz, Peter Igaz*

2nd Department of Medicine, Faculty of Medicine, Semmelweis University, Szentkirályi str. 46. H-1088 Budapest, Hungary

Received March 30, 2011; accepted April 18, 2011; Epub April 25, 2011; Published May 15, 2011

Abstract: Adrenocortical cancer is a rare tumor and its prognosis is poor. Although numerous tumor-associated genetic and signal transduction alterations have been described to date, its pathogenesis is still unclear. Hybridization-based DNA microarray approaches may reveal significant gene expression alterations and may thus contribute to a better understanding of tumorigenesis and may identify molecular markers applicable for the distinction of benign and malignant lesions. Beside gene expression patterns, studies on microRNAs seem to be useful, as well. Novel therapeutic targets might be established by these approaches. In this review, the authors attempt to summarize the main findings of mRNA and microRNA expression microarray studies performed to date in adrenocortical cancer including a recent meta-analysis of gene expression data and present novel pathogenic pathways.

Keywords: Adrenocortical tumor, gene expression, microRNA, microarray, meta-analysis

Introduction

Adrenocortical cancer (ACC) is a rare tumor with an incidence of 1-2/million/year [1]. Its prognosis is poor with a 5-year survival rate below 35% [2]. Most cases (60 %) are associated with clinically apparent hormone secretion (cortisol, androgens, very rarely aldosterone). Surgical tumor removal represents the most effective treatment option. Various chemotherapeutical protocols have been tested, but none of these turned out to be optimal. The adrenolytic agent mitotane is the only effective oral adjuvant medication. Adrenocortical cancer is not particularly sensitive to irradiation, but irradiation of the tumor bed can be proposed in patients with high risk of local recurrence [3].

Studies on ACC pathogenesis are important from a clinical point of view as they might reveal pathways including potential novel therapeutic targets. Several novel agents are being tested in various clinical trials that have been selected based on recent molecular studies.

Several genetic and signal transduction altera-

tions have been already described in ACC, but we are still far from a clear picture. Studies on rare monogenic disorders predisposing affected individuals to ACC are useful, as the pathomechanisms identified in these tumor syndromes have also been found in sporadic ACC. These tumor syndromes include the Li-Fraumeni syndrome, Beckwith-Wiedemann syndrome and familial adenomatous polyposis coli (FAP), whereas multiple endocrine neoplasia type 1 (MEN1), Carney-complex and McCune-Albright syndrome are mostly associated with benign adrenocortical tumors [4].

Li-Fraumeni syndrome is caused by mutations of the p53 tumor suppressor gene (*TP53*) and includes leukemia, breast cancer, soft tissue sarcoma and glioma beside ACC. The Beckwith-Wiedemann syndrome is characterized by Wilms's tumor, rhabdomyosarcoma, hepatoblastoma and adrenocortical tumors. Its pathogenesis involves the overexpression of insulin like growth factor 2 (IGF-2) mediated by disturbed genomic imprinting. Adrenocortical cancer has been described in rare cases of FAP that is characterized by the activation of the

Wnt/ β -catenin signal transduction pathway [4].

Somatic p53 mutations have been described mainly in advanced, large ACC tissues, and these therefore represent late events in ACC pathogenesis. Overexpression of IGF-2 is one of the most invariable findings in ACC [1]. Activation of the Wnt/ β -catenin signal transduction pathway is considered to be a definitive step in the pathogenesis in adrenocortical cancer [4].

Beside these major mechanisms, several other molecular alterations have been already described in ACC, as reviewed elsewhere [1, 2, 4]. In this review article, the authors focus on recent mRNA and microRNA profiling studies, including their own bioinformatics meta-analysis that have revealed several novel molecular alterations and pathways that seem to include novel biomarkers and also potential therapeutic targets.

Gene expression microarray studies in adrenocortical cancer

Gene expression microarray studies enable the simultaneous analysis of all genes in a given tissue. Several microarray studies have been already performed in adrenocortical cancer tissues that have resulted in major achievements including the establishment of novel biomarkers, tumor classification and novel pathogenic pathways.

Most studies examined adult adrenocortical tumors [5-13] and our review focuses on these.

The strongest signature identified is the malignancy signature that includes overexpression of IGF-2 in ACC [1, 14, 15]. Another major overexpressed gene is topoisomerase 2A (*TOP2A*). Overexpressed IGF-2 together with the proliferation marker Ki-67 might be used to differentiate benign and malignant tumors [12]. Several genes involved in cell cycle regulation including cyclins, cyclin dependent kinases etc. have been found to be overexpressed in ACC [15, 16].

de Fraipont et al. studied the gene expression patterns of 33 benign and 24 malignant tumors on custom microarrays [17]. They have identified two gene clusters, i.e. the steroidogenesis and the IGF-2 clusters which proved to be suitable to separate the benign and malignant tu-

mors. Malignant tumors have been characterized by overexpressed IGF-2 and underexpressed steroidogenesis cluster genes, whereas in benign tumors IGF-2 cluster genes were relatively underexpressed and steroidogenesis-related genes were overexpressed. The underexpression of genes involved in steroidogenesis has been described in several studies [15]. Moreover, 14 genes characteristic for tumor recurrence have also been identified in de Fraipont's study. These included genes important in immune regulatory processes (e.g. granzyme A, integrin β 2 and the interleukin 2 (IL2) receptor γ chain), which underlines the relevance of immune processes in the pathogenesis of ACC recurrence [17].

Among the other genes applicable for the differentiation of benign and malignant tumors, the underexpressed chromogranin-B (CgB) and transcription factor Egr1 should be listed [10]. Velazquez-Fernandez et al. [9] identified the significantly overexpressed ubiquitin-related genes USP4 and UFD1L in ACC, whereas the chemokine gene (*CXCL10*), cadherin 2 and several genes related to cell metabolism were down-regulated. In the study of Fernandez-Ranvier et al., the overexpression of the serotonin receptor 2B (*HTR2B*) and underexpression of cyclin B2 (*CCNB2*) and interleukin-13 receptor (*IL13RA2*) genes have been validated [5].

In line with previous studies demonstrating activation of the Wnt/ β -catenin signaling in ACC, several targets of this pathway have been found to be overexpressed in ACC [15].

Table 1 presents some important gene expression alterations identified in these studies.

In the studies by de Reyniès et al. [11] and Giordano et al. [8], subclassification of ACC based on gene expression patterns has been established: a subgroup with favorable and poor prognosis. Tumors in the poor prognosis group exhibited more advanced disease, high degree of mitoses [8] and an abundance of overexpressed cell cycle regulator genes [11]. Gene expression markers of recurrence free survival and overall survival have been identified in de Reyniès' study. Expression of *DLG7* (discs large homologue 7 *Drosophila*) and the *PINK1* (PTEN-induced putative kinase 1) were the most suitable for recurrence free survival, whereas *BUB1B* (bubbling uninhibited by benzimidazoles

mRNA and microRNA expression patterns in adrenocortical cancer

Table 1. Characteristics of some major gene expression alterations in selected microarray studies

Study	Distribution of samples	Genes overexpressed in ACC	Genes underexpressed in ACC
Giordano et al. [7]	3 NA, 4 ACA, 11 ACC	<i>IGF2, IGFBP3, TOP2A, CCNE1, UBCH10, CDK1, SPP1, IL13RA, HMGA1</i>	<i>ADH1, ADH2, TMOD, IGFBP5, IGFBP6, SDF1, CDKN1C, CYP11B1</i>
de Fraipont et al. [17]	33 ACA, 24 ACC	<i>IGF2, TGFβ2, FGFR1, FGFR4, MST1R, TGFB1, KCNQ1, KCNQ10T1, GAPD</i>	<i>StAR, CYP11A, HSD3B1, CYP11B1, CYP21A2, CYP17, PP1A, S100B, GPC3, INHA, CREM, RB1, NM23H5, TGFβ3</i>
Velázquez-Fernandez et al. [9]	13 ACA, 7 ACC	<i>USP4, UFD1L, IGF2, IGF2R, IGFBP3, IGFBP6, INPPL1, AQP3, H3F3B</i>	<i>ABCG1, CXCL10, RARRES2, ALDH1A1, CYBRD1, GSTA4, CDH2</i>
Slater et al. [10]	10 NA, 10 ACA, 10 ACC	<i>IGF2, CTSH, MCOLN3, FGFR1, AKR1C1, FN1</i>	<i>ALDH1A1, HSD3B1, FGF11, CgB, EGR1, MGC5306, CYFIP2, PCP4, QPCT, PALM, IL1R1</i>
West et al. [18]	7 NA, 5 ACA, 18 ACC (Childhood tumors)	<i>IGF2, TRIP, DLL3, HOXB13, CD22, CDK16, DUOX2,</i>	<i>PAH, HLA-DRA, HLA-DRB1, HLA-DPA1, APOE, PLAGL1, CYP11B1, NR4A1, NR4A2</i>
Fernandez-Ranvier et al. [5]	74 ACA, 11 ACC	<i>CCNB2, IL13RA2, CDCA7,</i>	<i>HTR2B, RARRES2, HSD3B2, ALDH1A1, SLC16A9</i>
Soon et al. [12]	6 NA, 16 ACA, 12 ACC	<i>IGF2, MAD2L1, CCNB1, CCNB2, ANLN, IGFBP3, TOP2A</i>	<i>ABLIM1, NAV3, SEPT4, RPRM, CYP11B1, H19, APOE, HSD3B2, ALDH1A1</i>
Fernandez-Ranvier et al. [6]	43 ACA, 11 ACC	Markers applicable for the establishment of malignancy: <i>SERPING1, MRPL48, TM7SF2, DDB1, NDUFS8, PRDX5</i>	
Giordano et al. [8]	10 NA, 22 ACA, 33 ACC	<i>IGF2, ANLN, CCNB2, CDC2, UBE2C, TOP2A, CDK1, IL13RA, IGFBP3, FGFR1, XPO1</i>	<i>H19, AADAC, HSD3B2, CYP11B1, KCNQ1, ADH1B, ALDH1A1, CDKN1C, IGFBP5, SERPING1</i>
de Reyniès et al. [11]	58 ACA, 34 ACC	Prognostic markers: <i>DLG7, PINK1, BUB1B</i>	

NA: normal adrenal cortex, ACA: benign adrenocortical adenoma, ACC: adrenocortical cancer

1 homolog beta) and *PINK 1* was found to be the most adequate marker or overall survival. It is interesting to note that all these genes encode proteins involved in cell cycle regulation [11].

There has been only a single study examining the transcriptome profile of childhood adrenocortical tumors [18]. Childhood ACC differs from its adult counterpart in many respects [19]. It has been hypothesized that childhood ACC originates from the abnormal presence or defective apoptosis of fetal adrenocortical cells [19]. Whereas somatic mutations of *TP53* (tumor protein 53) tumor suppressor gene are characteristic for advanced stages of adult ACC, germline mutations of *TP53* have been identified in a considerable proportion of childhood ACC cases. Increased dosage of *SF-1* (steroidogenic factor 1) and overexpressed *IGF-2* are also characteristic features of childhood ACC. In line with

the latter observation, overexpressed *IGF-2* has been described in West's microarray study together with the underexpression of the gene encoding the steroid biosynthetic enzyme *HSD3B2* (3 beta hydroxysteroid dehydrogenase type 2) and its transcriptional regulators *NR4A1* and *NR4A2* [18]. It is interesting to note that transcripts of major histocompatibility complex (MHC) genes have been found to be underexpressed in childhood ACC relative to benign tumors, again underlining the relevance of immune regulation in adrenocortical tumorigenesis [18, 19].

There are several similarities in the gene expression patterns between the fetal adrenal cortex and adrenocortical cancer. *IGF-2* functions as a growth factor of the fetal adrenal cortex. The underexpression of *HSD3B* and the *AKR1B1* (aldo-keto reductase family 1/1B) gene encoding an enzyme involved in steroid

hormone metabolism has been identified both in the fetal adrenal cortex and ACC [20].

Meta-analysis of publicly available and our own microarray data

Although there are several common observations, there are considerable differences in mRNA expression established in published microarray data. We have therefore hypothesized that by collecting, reclassifying and reanalyzing available gene expression data followed by pathway analysis, biologically relevant and previously unknown pathogenic pathways might be identified. Pathway analysis is a novel bioinformatical approach, which correlates user defined gene expression alterations to a continuously refreshed database that enables the network-based interpretation of differentially expressed genes.

We have recently analyzed the gene expression data of four pangenomic microarray studies [21]. We have reclassified and reanalyzed the data of altogether 164 tumors (97 benign and 67 malignant) and 18 normal adrenal cortex tissues. Beside these, we have also studied the significant gene lists of studies, where raw gene expression data have not been publicly available. We have attempted to establish correlations between gene expression alterations and chromosome aberrations by gene set enrichment analysis (GSEA) and leading edge analysis (LEA). Significant gene lists and chromosome aberrations of altogether 269 benign, 215 malignant tumors and 30 normal tissues have been analyzed in addition to the data retrieved from the four pangenomic studies. Gene expression alterations correlating with chromosome aberrations were subjected to Ingenuity Pathway Analysis (IPA). As studies on gene expression and chromosome aberrations (comparative genome hybridization, CGH) have been performed on distinct sample sets, we have performed a parallel gene expression microarray and CGH study on 11 tumor samples for controlling our approach. We have managed to correlate 46 out of 101 chromosome aberrations with significant gene expression alterations. Despite the low sensitivity (45.54 %), a rather high specificity (84.86 %) could be established.

Our preliminary analysis of biomarkers of malignancy in the meta-analysis has revealed that the combination of overexpressed anillin (*ANLN*)

and underexpressed serotonin receptor 2B (*HTR2B*) appeared to be the best predictor of malignancy. Underexpressed *HTR2B* has already been described and validated by Fernandez-Ranvier et al. [5], but the overexpression of *ANLN* is a novel finding. The actin-binding anillin is a protein involved in the regulation of cell division and its overexpression has been documented in a wide range of human tumors [22]. Further studies will be required to assess the applicability of these molecular markers.

Three major pathogenic pathways have been established both in our meta-analysis and in our own parallel mRNA expression-CGH study: i. damage of cell cycle, ii. retinoid signal transduction including the lipopolysaccharide/Toll like receptor 4 (TLR-4) pathway and iii. complement and antigen presentation [21].

Damage of cell cycle

Damage of cell cycle has been described in many tumors and in adrenocortical cancer, as well. Gene expression changes affecting both G1/S and G2/M transition have been described. Overexpression of cyclin E and cyclin dependent kinases (*CDK2*, *CDK4*) has been documented. We managed to correlate several gene expression alterations with chromosome aberrations. It is interesting to note that the protooncogene *c-myc*, whose overexpression is a common finding in several tumors, was found to be underexpressed in ACC and this could be correlated with the loss of 8q24, where its gene is harbored. By network topology modeling, underexpressed *c-myc* turned out to be a major (high-grade) node in the ACC gene expression network, and we hypothesize that it represents a major pathogenic event. Previous studies seem to confirm our finding, as *c-myc* was found to be underexpressed in ACC tissues by Northern-blotting [23]. Moreover, adrenocorticotropin (ACTH) administration to fetal adrenocortical cell cultures resulted in cellular differentiation and this was preceded by *c-myc* overexpression [24]. As described above, there are several similarities between the gene expression profiles of fetal adrenal cortex and ACC [20]. If overexpressed *c-myc* might induce differentiation, vice-versa, *c-myc* underexpression might be associated with increased proliferation in ACC. The adrenal cortex might therefore represent a unique cellular context, where in contrast to the majority of other tissues, *c-myc* underexpression

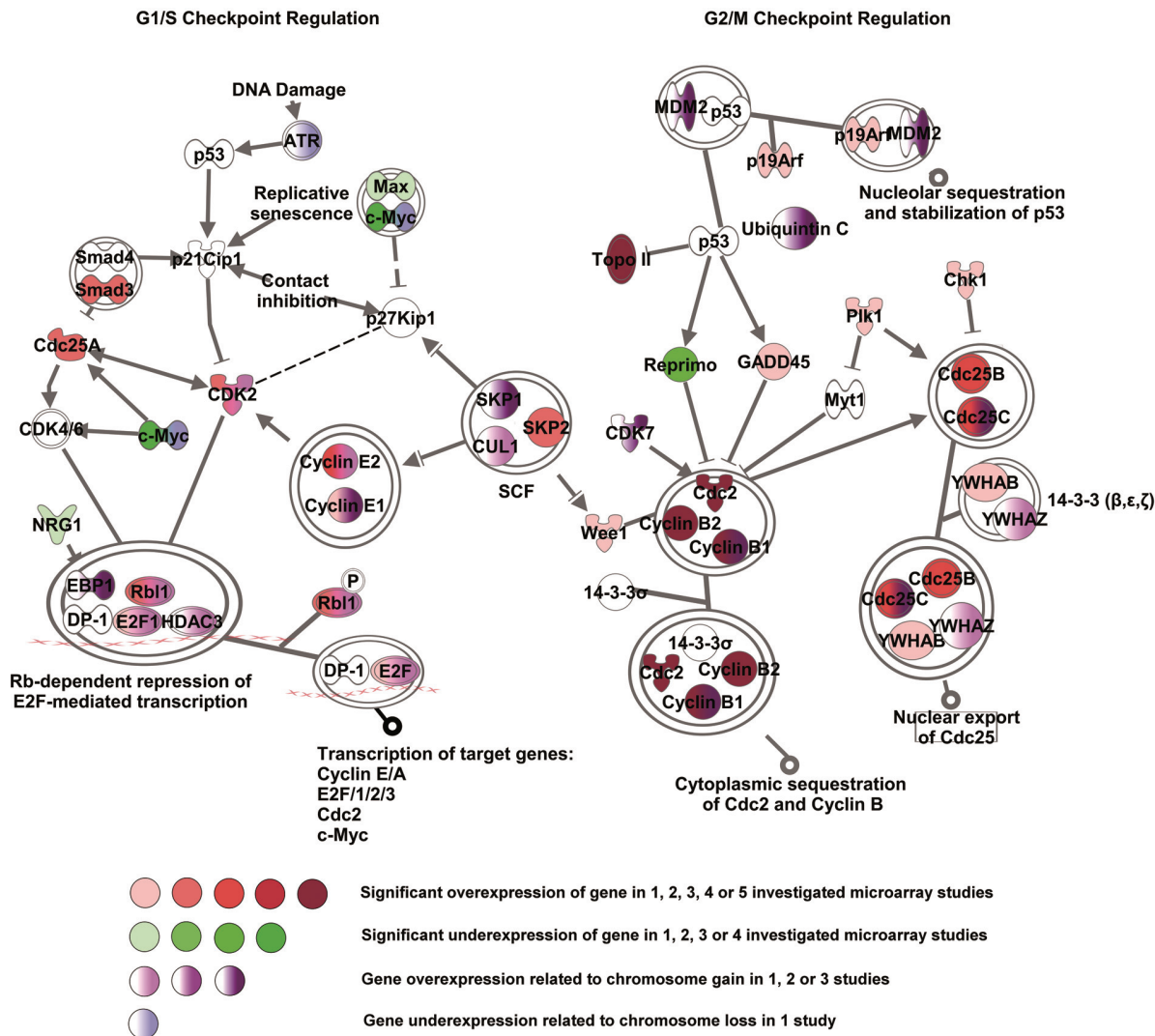


Figure 1. Results of pathway analysis (IPA) for the genes involved in cell cycle regulation. Adapted and reproduced with permission from Szabó PM et al., *Oncogene* (Nature Publishing Group), 2010 [21].

is associated with tumor growth.

Beside the overexpression of *TOP2A* described in many studies, we have noted the overexpression of exportin (*XPO1*). Exportin is involved in the cytoplasmic translocation of *TOP2A* that is implicated in the resistance of *TOP2A* to many cytostatic drugs also used in ACC therapy. Overexpression of exportin was established as a poor prognostic marker in ovarian cancer correlating with the cytoplasmic translocation of *TOP2A* and resistance to chemotherapy [25]. Such a mechanism may be present in ACC, as well, but further studies are needed for validation.

ing this hypothesis. **Figure 1** presents the alterations of genes involved in cell cycle regulation based on our meta-analysis.

Retinoic acid signaling

Retinoids are ligands of retinoic acid receptor (RAR) and retinoid x receptor (RXR). RAR binds all-trans retinoic acid and 9-cis retinoic acid, whereas RXR only binds 9-cis-retinoic acid. Retinoids are involved in the pathogenesis of many tumors and are used in the therapy of several tumors [26]. 9-cis-retinoic acid treatment of the human adrenocortical cancer cell line NCI-H25R

results in growth inhibition [27]. The healthy human adrenal cortex produces considerable amounts of retinoids, but the role of retinoids in normal adrenal physiology is unclear [28].

Results of our meta-analysis show that both the expression of receptors for retinoic acid and the production of retinoic acid are reduced in malignant adrenocortical tumours, thus both decreased biosynthesis and diminished action of retinoids might be implicated in adrenocortical tumorigenesis [21].

In our direct comparison of CGH and GSEA results, reduced expression of *RXRB* (RXR beta) could be correlated with the loss of chromosome 6q21 [21].

Retinoic acid bound to RXR forms heterodimers with other nuclear receptors. These include the liver X receptors (LXR). LXR was shown to be pivotal in the regulation of adrenocortical cholesterol metabolism and steroid hormone production [28]. We have noted underexpression of *LXRA* (LXR A receptor) that could be associated with the loss of 11q11. Peroxisome proliferator activator receptor gamma (PPARG) is also expressed in the adrenal cortex and it may also heterodimerize with RXR [29]. PPARG is involved in the regulation of glucose and lipid homeostasis and in the pathogenesis of several tumors [30]. Treatment of the NCI-H295R adrenocortical cancer cell line with PPARG agonist thiazolonediones inhibited its growth and promoted its differentiation in synergism with retinoids [27].

We have observed the underexpression of several genes involved in cholesterol and lipid metabolism. Based on our meta-analysis, we have raised the hypothesis that these gene expression changes are involved in the clinicopathological observation that malignant adrenocortical tumors are relatively lipid-poor in comparison with their benign counterparts.

The family of Toll like receptors (TLR) is linked to the interleukin 1 receptor (IL-1R) family and is involved in the regulation of inflammation and acute phase reaction. The activation of TLR4 by lipopolysaccharides (LPS) modulates retinoic acid signaling [31]. LPS directly stimulate cortisol secretion from NCI-H295R cells [32] and the expression of IL-6 and IL-8 in the normal human adrenal cortex [33]. We have noted the under-

expression of *TLR4* and the active *IL-1R1* receptor in our meta-analysis, whereas the expression of the decoy *IL-1R2* inhibiting IL-1 signal transduction was increased [21]. These gene expression alterations show the relevance of immune-neuroendocrine interactions and indicate the decreased activity of TLR4/IL1R system in adrenocortical tumors. **Figure 2** displays the gene expression alterations of retinoid signaling identified in our meta-analysis.

Complement system and antigen presentation

The third major pathway established by our meta-analysis was related to the complement system and antigen presentation. We have noted the underexpression of several members of the complement systems that involve several components of both classical and alternative pathways (e.g. complement C1q, C1QA and C1QB chains, H and D factors) [21].

Class II molecules of the major histocompatibility complex (MHC) are expressed in the normal human zona reticularis [34]. Malignant tumors lack MHCII expression, and therefore the detection of MHCII molecules is considered as a marker for benign adrenocortical lesions [35]. In our experimental system, decreased MHCII expression could be correlated with the loss of chromosome 6q21 [21].

Unfortunately, the pathogenic relevance of these observations is unclear, since there is only very few experimental evidence in this field.

In conclusion, three major pathogenic pathways have been established by our meta-analysis of available gene expression and cytogenetic data and our own experimental model on parallel gene expression and CGH profiling. These pathways include previously unknown pathogenic pathways and associated gene products might be used as novel diagnostic markers and therapeutic targets.

It is interesting to note that gene expression alterations established by previous studies as major events in adrenocortical tumorigenesis are not represented in these pathways. Although the overexpression of *IGF-2* is an invariable finding in all studies, including the studies used in our meta-analysis, it is absent from the three major pathways established in the meta-analysis. This observation might be explained

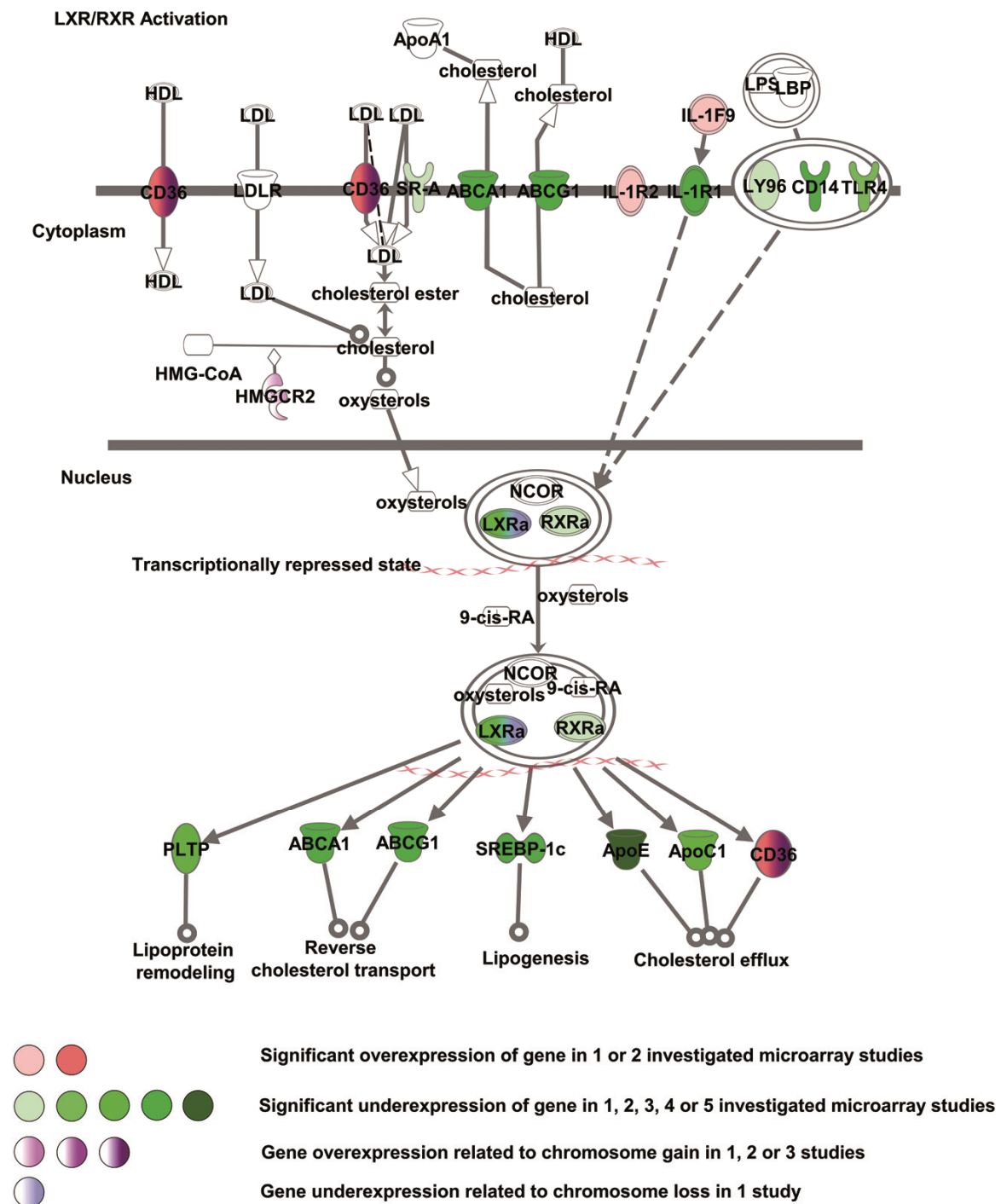


Figure 2. Pathways with altered gene expression patterns involved in LXR/RXR signalling in ACCs. Adapted and reproduced with permission from Szabó PM et al., *Oncogene* (Nature Publishing Group), 2010 [21].

that the bioinformatical pathway analysis represents a network-based novel approach and the established pathways might refer to “deeper”

tumorigenic pathways. Certainly, these pathways do not exclude the relevance of other major pathways.

MicroRNAs in adrenocortical cancer

MicroRNAs (miR) are short non-coding RNA molecules comprised of 20-24 nucleotides that are involved in the regulation of basic cellular processes (differentiation, proliferation, apoptosis). As the endogenous mediators of RNA interference, they can specifically bind to the non-translated 3' regions (UTR: untranslated region) of target mRNA molecules inducing their degradation or the inhibition of translation. MicroRNAs have been shown to be involved in the regulation of several homeostatic processes e.g. development of adipocytes, regulation of insulin secretion, immune functioning etc [36-38].

The altered expression of microRNAs have been described in several diseases but most data are related to their roles in tumorigenesis. Different expression profiles of microRNAs can be exploited in the distinction of benign and malignant tumors that could be of great relevance for tumors where the histological diagnosis of malignancy is difficult [39]. Follicular tumors of the thyroid and adrenocortical tumors also belong to this category [1, 40]. Analysis of microRNA expression patterns may even be advantageous over mRNA gene expression profiles (transcriptomics), since there are much fewer microRNAs (approximately 1000 in humans) than mRNAs and therefore fewer and better distinguished tumor classes might be established [41].

The gene expression patterns of adrenocortical tumors have been examined in four studies to date (3 on adult and 1 on childhood tumors) [13, 42-44].

In our study, samples from 10 normal adrenal cortex, 10 hormonally inactive adenomas, 9 cortisol-producing adenomas and 7 cortisol-producing malignant tumors have been studied [13]. 22 significantly differentially expressed microRNAs have been identified, and 6 of these were validated by real time RT-PCR. Three miRs (*miR-503*, *miR-184* and *miR-210*) were overexpressed, whereas three others (*miR-214*, *miR-511* and *miR-375*) were underexpressed in ACC relative to benign tumors and the normal cortex. Overexpressed miRs can be regarded as oncogenic, whereas underexpressed as tumor suppressors [45]. Expression difference of *miR-503* and *miR-511* (delta cycle time Δ CT: $dCT_{miR511} - dCT_{miR503}$) can be used for the differentiation of benign and malignant tumors with 100 %

sensitivity and 93 % specificity. Considering the difficulties in the histological analysis of adrenocortical tumors, this marker of malignancy might be of major practical relevance. By the bioinformatical analysis of predicted miR target mRNAs, damage of cell cycle G2-M checkpoint has been identified as a major, miR-mediated pathogenic pathway [13].

In a subsequent study, 17 adenoma and 22 carcinoma samples have been studied [42]. 23 significantly differentially expressed miRs have been identified. Significant differences in expression have been validated by qRT-PCR for *miR-7*, *miR-195*, *miR-335* and *miR-483-5p*. *miR-195* and *miR-335* have been significantly underexpressed, whereas *miR-483-5p* has been overexpressed in malignant tumors. The *miR-7* was significantly underexpressed in ACC relative to benign adenomas and normal tissues. Underexpression of *miR-195* and overexpression of *miR-483-5p* could be correlated with a worse prognosis. In a most recent study on a larger tumor set, the overexpression of *miR-483-5p* has been confirmed, and the underexpression of *miR-100*, *miR-125b* and *miR-195* has been validated. *miR-483-5p* seemed to have both a high positive (100 %) and negative predictive value (92%). It is interesting to note that the gene for *miR-483-5p* has been mapped to intron 2 of the IGF-2 gene and its overexpression might be therefore related to IGF-2 overexpression [43].

In the study by Doghman et al. on childhood adrenocortical tumors, 26 significantly differentially expressed microRNAs have been identified [44]. The significantly underexpressed *miR-99a* and *miR-100* share the same seed sequence and are predicted to target components of insulin like growth factor receptor 1 (IGFR1), mammalian target of rapamycin (mTor) and raptor signaling. By blocking mTor signaling both in vitro and in vivo xenograft models, adrenocortical tumor growth could be inhibited that raises the possibility that mTor inhibitors might be effective in ACC chemotherapy [44].

Concluding remarks

Studies on microarray and microRNA expression profiling have revealed novel aspects of adrenocortical cancer pathogenesis. These observations have already paved the way for novel tumor classifications and molecular markers of malignancy. Since the options for drug therapy

in ACC are rather limited, potential novel therapeutic targets established by these studies may be relevant for future targeted ACC therapy [46]. As there are still considerable differences in the significant gene lists established by various studies, approaches involving meta-analysis of microarray data may be of great help for the comprehensive interpretation of data.

Please address correspondence to: Peter Igaz, MD, PhD, 2nd Department of Medicine, Faculty of Medicine, Semmelweis University, H-1088 Budapest, Szentkirályi str. 46., Hungary. Tel: +36-1-4591500, Fax: +36-1-2660816, E-mail: igapet@bel2.sote.hu

References

- [1] Igaz P, Wiener Z, Szabó P, Falus A, Gaillard RC, Horányi J, Rácz K, Tulassay Z. Functional genomics approaches for the study of sporadic adrenal tumor pathogenesis: Clinical implications. *J Steroid Biochem Mol Biol* 2006; 101: 87-96.
- [2] Libé R, Bertherat J. Molecular genetics of adrenocortical tumours, from familial to sporadic diseases. *Eur J Endocrinol* 2005; 153: 477-487.
- [3] Fassnacht M, Allolio B. Clinical management of adrenocortical carcinoma. *Best Pract Res Clin Endocrinol Metab* 2009; 23: 273-289.
- [4] Bertherat J, Bertagna X. Pathogenesis of adrenocortical cancer. *Best Pract Res Clin Endocrinol Metab* 2009; 23: 261-271.
- [5] Fernandez-Ranvier GG, Weng J, Yeh RF, Khanafshar E, Suh I, Barker C, Duh QY, Clark OH, Kebebew E. Identification of biomarkers of adrenocortical carcinoma using genomewide gene expression profiling. *Arch Surg* 2008; 143: 841-846.
- [6] Fernandez-Ranvier GG, Weng J, Yeh RF, Shibru D, Khafnashar E, Chung KW, Hwang J, Duh QY, Clark OH, Kebebew E. Candidate diagnostic markers and tumor suppressor genes for adrenocortical carcinoma by expression profile of genes on chromosome 11q13. *World J Surg* 2008; 32: 873-881.
- [7] Giordano TJ, Thomas DG, Kuick R, Lizyness M, Misek DE, Smith AL, Sanders D, Aljundi RT, Gauger PG, Thompson NW, Taylor JM, Hanash SM. Distinct transcriptional profiles of adrenocortical tumors uncovered by DNA microarray analysis. *Am J Pathol* 2003; 162: 521-531.
- [8] Giordano TJ, Kuick R, Else T, Gauger PG, Vinco M, Bauersfeld J, Sanders D, Thomas DG, Doherty G, Hammer G. Molecular classification and prognostication of adrenocortical tumors by transcriptome profiling. *Clin Cancer Res* 2009; 15: 668-676.
- [9] Velázquez-Fernandez D, Laurell C, Geli J, Höög A, Odeberg J, Kjellman M, Lundeberg J, Hamberger B, Nilsson P, Backdahl M. Expression profiling of adrenocortical neoplasms suggests a molecular signature of malignancy. *Surgery* 2005; 138: 1087-1094.
- [10] Slater EP, Diehl SM, Langer P, Samans B, Ramaswamy A, Zielke A, Bartsch DK. Analysis by cDNA microarrays of gene expression patterns of human adrenocortical tumors. *Eur J Endocrinol* 2006; 154: 587-598.
- [11] de Reyniès A, Assié G, Rickman DS, Tisser F, Groussin L, René-Corail F, Dousset B, Bertagna X, Clauser E, Bertherat J. Gene expression profiling reveals a new classification of adrenocortical tumors and identifies molecular predictors of malignancy and survival. *J Clin Oncol* 2009; 27: 1108-1115.
- [12] Soon PS, Gill AJ, Benn DE, Clarksom A Robinson BG, McDonald KL, Sidhu SB. Microarray gene expression and immunohistochemistry analyses of adrenocortical tumors identify IGF2 and Ki-67 as useful in differentiating carcinomas from adenomas. *Endocr Relat Cancer* 2009; 16: 573-583.
- [13] Tömböl Z, Szabó PM, Molnár V, Wiener Z, Tölgyesi G, Horányi J, Riesz P, Reismann P, Patócs A, Likó I, Gaillard RC, Falus A, Rácz K, Igaz P. Integrative molecular bioinformatics study of human adrenocortical tumors: micro RNA, tissue-specific target prediction, and pathway analysis. *Endocr Relat Cancer* 2009; 16: 895-906.
- [14] Soon PS, McDonald KL, Robinson BG, Sidhu SB. Molecular markers and the pathogenesis of adrenocortical cancer. *Oncologist* 2008; 13: 548-561.
- [15] Ragazzon B, Assié G, Bertherat J. Transcriptome analysis of adrenocortical cancers: from molecular classification to the identification of new treatments. *Endocr-Relat Cancer* 2011; 18: R15-R27.
- [16] Bourcigaux N, Bourcigaux N, Gaston V, Logié A, Bertagna X, Le Bouc Y, Gicquel C. High expression of cyclin E and C1 CDK and loss of function of p57KIP2 are involved in proliferation of malignant sporadic adrenocortical tumors. *J Clin Endocrinol Metab* 2000; 85: 322-330.
- [17] de Fraipont F, El Atifi M, Cherradi N, Le Moigne G, Defaye G, Houlgatte R, Bertherat J, Bertagna X, Plouin PF, Baudin E, Berger F, Gicquel C, Chabre O, Feige JJ. Gene expression profiling of human adrenocortical tumors using complementary deoxyribonucleic Acid microarrays identifies several candidate genes as markers of malignancy. *J Clin Endocrinol Metab* 2005; 90: 1819-1829.
- [18] West AN, Neale GA, Pounds S, Figueredo BC, Rodriguez Galindo C, Pianovski MA, Oliveira Filho AG, Malkin D, Lalli E, Ribeiro R, Zambetti GP. Gene expression profiling of childhood adrenocortical tumors. *Cancer Res* 2007; 67: 600-608.
- [19] El Wakil A, Doghman M, Latre de Late P,

- Zambetti GP, Figueiredo BC, Lalli E. Genetics and genomics of childhood adrenocortical tumors. *Mol Cell Endocrinol* 2010; doi:10.1016/j.mce.2010.11.008.
- [20] Coulter CL. Fetal adrenal development: insight gained from adrenal tumors. *Trends Endocrinol Metab* 2005; 16: 235-242.
- [21] Szabó PM, Tamási V, Molnár V, Andrásfalvy M, Tömböl Z, Farkas R, Kövesdi K, Patócs A, Tóth M, Szalai C, Falus A, Rácz K, Igaz P. Meta-analysis of adrenocortical tumor genomics data: novel pathogenic pathways revealed. *Oncogene* 2010; 29: 3163-3172.
- [22] Hall PA, Todd CB, Hyland PL, McDade SS, Grabsch H, Dattani M, Hillan KJ, Russell SE. The septin-binding protein anillin is overexpressed in diverse human tumors. *Clin Cancer Res* 2005; 11: 6780-6786.
- [23] Liu J, Voutilainen R, Kahri AI, Heikkilä P. Expression patterns of the c-myc gene in adrenocortical tumors and pheochromocytomas. *J Endocrinol* 1997; 152: 175-181.
- [24] Heikkilä P, Arola J, Salmi A, Kahri AI. ACTH-induced c-myc proto-oncogene expression precedes antimitogenic effect during differentiation of fetal rat adrenocortical cells. *J Endocrinol* 1995; 145: 379-385.
- [25] Faggad A, Darb-Esfahani S, Wirtz R, Sinn B, Sehoul J, Könsgen D, Lage H, Weichert W, Noske A, Budczies J, Müller BM, Buckendahl AC, Röske A, Eldin Elwali N, Dietel M, Denkert C. Topoisomerase IIalpha mRNA and protein expression in ovarian carcinoma: correlation with clinicopathological factors and prognosis. *Mod Pathol* 2009; 22: 579-588.
- [26] Shimizu M, Takai K, Moriwaki H. Strategy and mechanism for the prevention of hepatocellular carcinoma: phosphorylated retinoid X receptor alpha is a critical target for hepatocellular carcinoma chemoprevention. *Cancer Sci* 2009; 100: 369-374.
- [27] Ferruzzi P, Ceni E, Tarocchi M, Grappone C, Milani S, Galli A, Fiorelli G, Serio M, Mannelli M. Thiazolidinediones inhibit growth and invasiveness of the human adrenocortical cancer cell line H295R. *J Clin Endocrinol Metab* 2005; 90: 1332-1339.
- [28] Cummins CL, Volle DH, Zhang Y, McDonald JG, Sion B, Lefrançois-Martinez AM, Caira F, Veysière G, Mangelsdorf DJ, Lobaccaro JM. Liver X receptors regulate adrenal cholesterol balance. *J Clin Invest* 2006; 116: 1902-1912.
- [29] Betz MJ, Shapiro I, Fassnacht M, Hahner S, Reincke M, Beuschlein F. Peroxisome proliferator-activated receptor-gamma agonists suppress adrenocortical tumor cell proliferation and induce differentiation. *J Clin Endocrinol Metab* 2005; 90: 3886-3896.
- [30] Ondrey F. Peroxisome proliferator-activated receptor gamma pathway targeting in carcinogenesis: implications for chemoprevention. *Clin Cancer Res* 2009; 15: 2-8.
- [31] Ghose R, Zimmerman TL, Thevananther S, Karpen SJ. Endotoxin leads to rapid subcellular re-localization of hepatic RXRalpha: A novel mechanism for reduced hepatic gene expression in inflammation. *Nucl Recept* 2004; 2: 4.
- [32] Vakharia K, Hinson JP. Lipopolysaccharide directly stimulates cortisol secretion by human adrenal cells by a cyclooxygenase-dependent mechanism. *Endocrinology* 2005; 146: 1398-402.
- [33] Kanczkowski W, Zacharowski K, Wirth MP, Ehrhart-Bornstein M, Bornstein SR. Differential expression and action of Toll-like receptors in human adrenocortical cells. *Mol Cell Endocrinol* 2009; 300: 57-65.
- [34] Wolkersdörfer GW, Marx C, Brown J, Schröder S, Füssel M, Rieber EP, Kuhlisch E, Ehninger G, Bornstein SR. Prevalence of HLA-DRB1 genotype and altered Fas/Fas ligand expression in adrenocortical carcinoma. *J Clin Endocrinol Metab* 2005; 90: 1768-1774.
- [35] Marx C, Wolkersdörfer GW, Brown JW, Scherbaum WA, Bornstein SR. MHC class II expression – a new tool to assess dignity in adrenocortical tumours. *J Clin Endocrinol Metab* 1996; 81: 4488-4491.
- [36] He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet* 2004; 5: 522-531.
- [37] Cuellar TL, McManus MT. MicroRNAs and endocrine biology. *J Endocrinol* 2005; 187: 327-332.
- [38] O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol* 2010; 10: 111-122.
- [39] Garzon R, Fabbri M, Cimmino A, Calin GA, Croce CM. MicroRNA expression and function in cancer. *Trends Mol Med* 2006; 12: 580-587.
- [40] Weber F, Teresi RE, Broelsch CE, Frilling A, Eng C. A limited set of human microRNA is deregulated in follicular thyroid carcinoma. *J Endocrinol* 2006; 91: 3584-3591.
- [41] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. *Nature* 2005; 435: 834-838.
- [42] Soon PS, Tacon LJ, Gill AJ, Bambach CP, Sywak MS, Campbell PR, Yeh MW, Wong SG, Clifton-Bligh RJ, Robinson BG, Sidhu SB. MiR-195 and miR-483-5p identified as predictors of poor prognosis in adrenocortical cancer. *Clin Cancer Res* 2009; 15: 7684-7692.
- [43] Patterson EE, Holloway AK, Weng J, Fojo T, Kebebew E. MicroRNA profiling of adrenocortical tumors reveals miR-483 as a marker of malignancy. *Cancer* 2011; DOI: 10.1002/cncr.25724.
- [44] Doghman M, El Wakil A, Cardinaud B, Thomas E, Wang J, Zhao W, Peralta-Del Valle MHC,

- Figueiredo BC, Zambetti GP, Lalli E. Regulation of insulin-like growth factor-mammalian target of rapamycin signaling by microRNA in childhood adrenocortical tumors. *Cancer Res* 2010; 70 (11): 4666-75.
- [45] Chen CZ. MicroRNAs as oncogenes and tumor suppressors. *N Engl J Med* 2006; 353: 1768-1771.
- [46] Maluf DF, de Oliveira DH, Lalli E. Therapy of adrenocortical cancer: present and future. *Am J Cancer Res* 2011;1(2):222-232.