Journal of the Arkansas Academy of Science

2012

Analysis of Human TACC3 in Cellular Responses to Polycyclic Aromatic Hydrocarbons

I. H. Still
Arkansas Tech University, istill@atu.edu

B. Lauffart
Arkansas Tech University

Follow this and additional works at: http://scholarworks.uark.edu/jaas

Part of the Cancer Biology Commons

Recommended Citation

Analysis of Human TACC3 in Cellular Responses to Polycyclic Aromatic Hydrocarbons

I.H. Still[1*] and B. Lauffart[2]

Departments of Biological[1] and Physical Sciences[2], Arkansas Tech University, 1701 N. Boulder Avenue, Russellville, AR 72802

*Correspondence to istill@atu.edu

Abstract

Very little is known about the etiology of ovarian cancer. However, studies have shown that occupational exposure to polycyclic aromatic hydrocarbons (PAHs) and tobacco smoking can increase ovarian cancer risk. Previously, we have determined that 100% of ovarian tumors show loss or aberrant subcellular localization of TACC3 (transforming acidic coiled coil 3) relative to the normal ovarian surface epithelium (OSE). In mice, a role for TACC3 in the cellular response to PAH has been defined. However, comparable studies for human TACC3 has not been performed. In this report, we show that specific knockdown of TACC3 in human ovarian cancer cells increases the basal level, and distorts the PAH-induced expression, of genes involved in metabolizing the procarcinogen PAH benzo[a]pyrene to its DNA damaging epoxide. In addition, we demonstrate that PAH stabilizes the TACC3 protein and induces the export of TACC3 and one of its binding partners, Ku70, from the nucleus. This parallels the subcellular distribution of TACC3 in those ovarian cancers that express TACC3. These data suggest that functional downregulation of TACC3 could aid tumor progression by altering cellular responses to chemical carcinogens and the DNA damage that they induce.

Introduction

Ovarian cancer accounts for 4-6% of all cancers in women, but has an overall survival rate of less than 40%, a higher mortality than any other gynecological malignancy (Greenlee et al 2000). In part, this low survival rate is due to our poor understanding of the mechanisms underlying initiation and progression of this cancer and thus efficient prevention and adequate early detection. While BRCA1 and BRCA2 mutations account for 35-50% of familial ovarian cancer (Gayther et al 1999, Werness et al 2000), the etiological basis of sporadic ovarian cancer is still unclear, with no single high penetrance gene defect significantly associated with the initiation of the sporadic cancer. Instead, it is likely that, as with most human cancer, ovarian cancer is due to the poorly understood interactions between environmental insults and low penetrance genetic factors.

Occupational exposure to complex organic compounds, such as PAHs, is associated with up to 4-fold elevated risk of ovarian cancer (Shields et al 2002, Langseth et al 2004, Guo et al 2004). However, PAHs are also common environmental pollutants, chiefly as products of incomplete combustion, being found in charred food, fossil fuel exhaust and cigarette smoke. Benzo[a]pyrene (B[a]P) is one of the most prevalent PAH in tobacco smoke, and studies have linked cigarette smoking with an increased risk of developing several cancers, including forms of ovarian cancer (Green et al 2001, Terry et al 2003, Pan et al 2004, Zhang et al 2004, Niwa et al 2005, Jordan et al 2006, Rossing et al 2008).

The normal mechanism for clearance of PAHs requires the metabolism of the hydrophobic compound to a less toxic and more water soluble metabolite that can be excreted from the body (Jakoby et al 1982). Phase I metabolic enzymes are responsible for the initial hydroxylation or metabolic reduction of the hydrocarbon. The genes encoding these enzymes, such as CYP1A1 and CYP1B1, are induced by the liganded arylhydrocarbon receptor (AhR), heterodimerized to the AhR-nuclear translocator (ARNT) protein (Zhang et al 1998, Tsuchiya et al 2003). However, for some species of PAH, Phase I metabolism results in the activation of the initial PAH to a more potent and chemically active compound. For instance, the prototypical PAH, B[a]P, can be activated, first to the 7,8-epoxide intermediate, and then to the corresponding diol-9,10-epoxide (BPDE) (Harvey 1991). Failure to clear these reactive epoxides can then result in the formation of DNA adducts, which are normally identified and removed by DNA damage response and repair pathways. The major pathway responsible for the repair of PAH-damaged DNA is nucleotide excision repair (NER) (Yang et al 1982,

Analysis of Human TACC3 in Cellular Responses to Polycyclic Aromatic Hydrocarbons

MacLeod et al 1991), with double-strand DNA break (DSB) repair and mismatch repair (MMR) playing auxiliary roles (Toyooka et al 2004, Wu et al 2003). However, such repair can be further compromised, as the epoxides themselves are potent inhibitors of estrogen regulated BRCA1 expression (Jeffy et al 1999, 2002). Thus, the accumulation and incorrect repair of PAH-DNA adducts can lead to mutations and genetic damage involving homologous and non-homologous recombination (Abe & Sasaki 1982, Sasaki 1982), thereby increasing ovarian cancer risk.

Several cancers, in particular, breast, ovarian, endometrial and cervical cancers exhibit loss of chromosome 4p16 (Forozan et al 2000, Ramus et al 2003, Sherwood et al 2000, Suzuki et al 2000, Wang et al 2004), the site of the TACC3 gene (see Eslinger et al 2009 for review). We have found 1) that 100% of ovarian tumors show loss or aberrant subcellular localization of TACC3, 2) constitutional mutations in the TACC3 gene in patients with ovarian cancer, in the absence of mutations in known predisposition genes and 3) several novel coding substitutions in sporadic ovarian tumors that could interfere with posttranslational modifications and interactions that govern TACC3 function (see Eslinger et al 2009 and references therein). Combined, these data suggest that the functional inactivation and/or dysregulation of TACC3 plays a pivotal role in malignant progression in a significant number of ovarian tumors.

In the mouse, TACC3 is involved in the cellular response to PAH through a direct interaction with the ARNT1 protein (Sadek et al 2000). However, similar analyses of the human TACC3 protein have not been carried out. In this manuscript, we examine the potential role of TACC3 in responses to PAH in human ovarian cancer. We show that specific knockdown of human TACC3 in ovarian cancer cells increases the basal transcriptional level of genes involved in metabolizing B[a]P, however TACC3 knockdown also reduces the usual induction of the Phase I enzyme CYP1A1. PAH also induces a partial redistribution of TACC3 from the nucleus to the cytoplasm. These data suggest that the functional downregulation of TACC3 observed in most ovarian tumors aids tumor progression by altering cellular responses to chemical carcinogens and the DNA damage that they induce.

Materials and Methods

Cell lines and immunological procedures

A2780, SkOV3 and HeLa cells were obtained from ATCC and maintained in DMEM +10% fetal calf serum containing the antimycotic/antibiotic normocin (Invivogen, USA). α-TACC 3 antibody was described in Gangisetty et al (2004). Secondary antibodies and conjugates were obtained from Jackson Laboratories.

For protein extraction, cells were washed with ice-cold 1xPBS. Nuclear and cytoplasmic extracts were prepared according to the protocol of Schreiber et al (1989), while whole cell lysates were prepared as previously described in Lauffart et al (2007b). Proteins were separated by 8% w/v SDS-PAGE and immunoblotted with respective antibodies as described in Lauffart et al (2007b).

siRNA-mediated downregulation of TACC3 and semi quantitative rt-PCR analysis

The 21 nucleotide siRNA used to target human TACC3 was previously described and used in Lauffart et al (2007a). siRNA controls were obtained from Dharmacon, USA. All siRNAs were manipulated according to manufacturer's instructions. Briefly, cells were plated in triplicate into 24 well plates. After attachment, the cells were treated with 100nM siRNA to TACC3 or 100nM control siRNA (Dharmacon, USA) in the presence of 5nM siGLO (transfection efficiency control) for 48hrs prior to further treatment with PAH, and/or harvest. cDNA was prepared from 1μg of RNA using the iScript cDNA synthesis system (BioRAD, USA), according to manufacturer's instructions. Expression of a selection of the PAH response battery, cytochrome P450s CYP1A1 and CYP1B1, NADPH dehydrogenase, quinone 1 (NQO1) and aldehyde dehydrogenase 3c (ALDH3c) was analyzed by semiquantitative reverse transcription-polymerase chain reaction (rt-PCR). Actin was used as a quantification control. Amplification primers were selected from adjacent exons in each gene to readily detect any genomic contamination (sequences and amplification conditions are available upon request). PCR amplification was performed for 25 cycles and repeated three times. All results shown were obtained from the same experiment, but similar results were obtained in the replicates.

Results and Discussion

The ARNT1 binding region located in the TACC domain of TACC3 is conserved between species (Eslinger et al 2009). Thus, due to this degree of conservation, we predicted that human TACC3 has a functional role in the transcriptional regulation of PAH responsive genes such as CYP1A1 and CYP1B1. We tested this hypothesis by examining the effect of

siRNA-mediated inhibition of endogenous TACC3 in two ovarian cell lines A2780 and SkOV3, and the cervical cancer cell line HeLa. As shown in Fig. 1A, transient transfection of a TACC3 siRNA, increased the constitutive expression level of CYP1B1 in A2780 and SkOV3. Induction of two other genes in the PAH "response battery", NQO1 and ALDH3c was also noted, in the absence of PAH. Intriguingly, this effect was specific to the ovarian cancer cell lines, and was not observed in the cervical cancer cell line, HeLa. In SkOV3, a complete knockdown of endogenous human TACC3 expression resulted in induction of CYP1A1 in the absence of B[a]P, which then only increased an
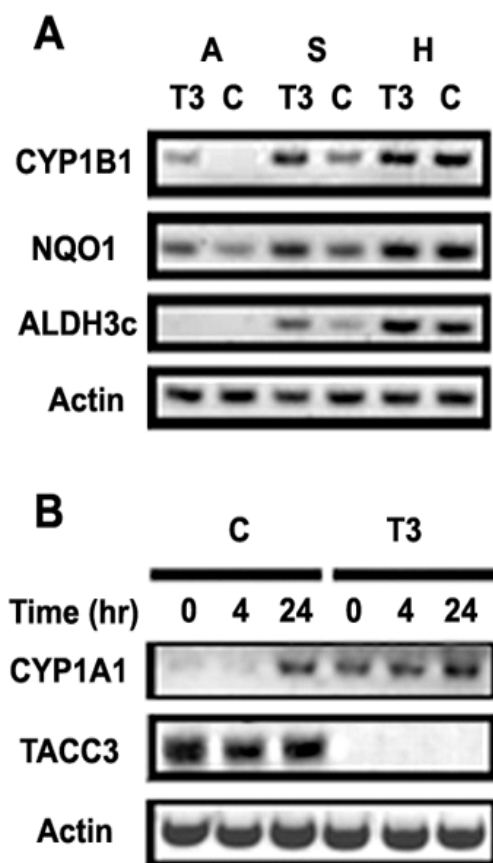

Figure 1: siRNA-mediated downregulation of TACC3 increases the base level of PAH response and metabolizing genes (**A**), but reduces transcriptional induction upon PAH exposure (**B**). (**A**): A2780 (A), SkOV3 (S) or HeLa (H) cells were treated with siRNA to TACC3 (T3) or control siRNA (C) (Dharmacon, USA) for 48hrs prior to harvest (see Materials and Methods). Semiquantitative rt-PCR was performed as detailed in Materials and Methods. Basal levels of Cyp1B1, Nqo1 and Aldh3c increased in the ovarian cancer cell lines, but not the cervical cancer cell line HeLa. (**B**): Effect of TACC3 knockdown on PAH-induced expression of CYP1A1 in SkOV3. Experimental procedures were as described in (A), however, prior to harvest, the transfected cells were treated with 5μM B[a]P for the indicated times.

additional two fold after exposure to the PAH for 24 hours (Fig. 1B). Intriguingly, these findings recapitulate the observation that many ovarian tumors express high levels of CYP1A1 and CYP1B1, even in the absence of PAH stimulation (Leung et al 2005, McFadyen et al 2001). These results suggest that TACC3 can act as both a transcriptional repressor in the absence of PAH and an activator when PAH is present. This type of effect is known for several transcriptional regulators. Thus, TACC3 may play a similar function to the FHL and FOG families [with which it also interacts (Garriga-Canut and Orkin 2004, Lauffart et al 2007b)] acting as either a corepressor or coactivator, depending upon the context of the promoter and the availability of ligand [similar to PGC-1 (Tcherepanova et al 2000, Delerive et al 2002)]. Alternatively, TACC3 may act as a promoter-specific transcriptional switch, changing from a transcriptional repressor to an activator, as noted for TIF1, and CBF-1/RBP-J in the Notch signaling pathway (Kao et al 1998). Previously characterized TACC3 interactions suggest that either mechanism is possible as TACC3 directly binds to methyl-binding protein (MBD) at methylated promoters (Angrisano et al 2006) and histone acetyltransferases (Gangisetty et al 2004). Thus, TACC3 may normally stabilize histone deacetylases bound to methylated promoters, such as that of uninduced CYP1A1 (Schnekenburger et al 2007), but then bring in histone acetyltransferases, triggering activation of the previously methylated promoter when the cell is exposed to PAH. Consequently, the observed loss of TACC3 from the nucleus in ovarian tumors (Lauffart et al 2005) could be associated with abnormalities in PAH metabolism in the OSE and its malignant derivatives.

Dynamic posttranslational modifications by ubiquitin regulate the structural integrity and stability of several proteins involved in the response to PAH and the genetic damage that they induce (Ma & Baldwin 2000, Glockzin et al 2003, Ng et al 2003). Furthermore, based upon current interactome data, TACC proteins are predicted to interact with ubiquitin conjugating enzymes (Lauffart et al 2007a). To determine whether PAH induces changes in TACC3 stability via the ubiquitin-proteasome pathway, parallel cultures of the ovarian cancer cell lines A2780 and SkOV3 were treated with B[a]P in the presence of the 26S proteasome inhibitor MG132. In addition, the cells were treated with a combination of MG132 and BP. As shown in Fig. 2, B[a]P and MG132 individually increase the steady state level of the TACC3 protein. The combination of the two reagents produced a

Analysis of Human TACC3 in Cellular Responses to Polycyclic Aromatic Hydrocarbons
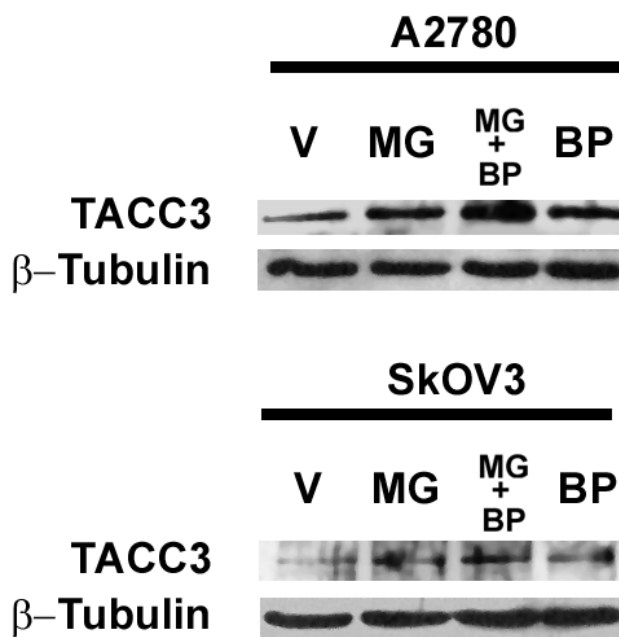

Figure 2: Treatment of ovarian cancer cells with benzo[a]pyrene stabilizes endogenous TACC3. Cells were treated for 6hr with DMSO (V), 20μM MG132 (MG), 5μM benzo[a]pyrene (BP) or a combination of MG132 and benzo[a]pyrene (MG+BP). 10μg of whole cell extract was immunoblotted, and incubated with α-TACC3 antisera. β-Tubulin was used to demonstrate equivalent loading of samples.
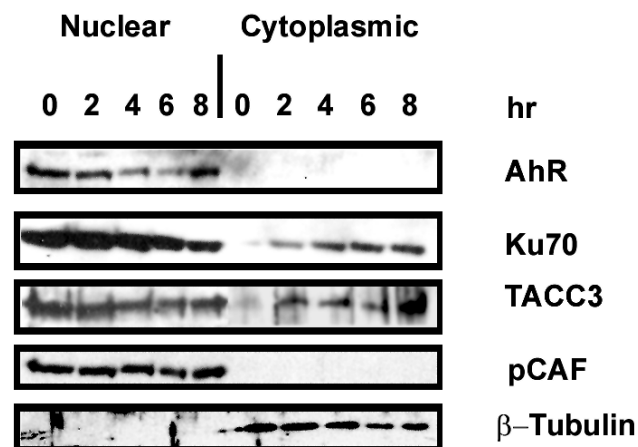

Figure 3: Benzo-[a]-pyrene causes TACC3 and Ku70 to exit the nucleus. A2780 ovarian cancer cells were treated with B[a]P for 0-8 hours. Nuclear and cytoplasmic extracts were isolated from treated cells and electrophoresed as described in Materials and Methods. pCAF and β-tubulin were used as loading control and to test for successful separation of nuclear vs. cytoplasmic fractions.

synergistic effect, which is more pronounced in A2780, than SkOV3. Recently, two sites in TACC3, K562 and K673, have been identified as ubiquitinylation targets in human embryonic kidney, HEK293T, cells and MV4–11 acute monocytic leukemia cells grown under standard conditions, although neither site was regulated by MG-132 (Wagner et al 2011). However, the data from the two ovarian cancer cell lines presented here suggests that in response to PAH exposure, TACC3 may be protected from proteolytic degradation by the 26S proteasome, potentially enhancing TACC3's capability to induce PAH response genes.

In order for TACC3 to affect transcription of PAH response genes, logically TACC3 must be located in the nucleus. Indeed, TACC3 is located in the nuclei of normal ovarian surface epithelial cells (Lauffart et al 2005). To further confirm that this was also the case in the ovarian cancer cell lines that were used in this study, we isolated nuclear and cytoplasmic extracts from A2780 and SkOV3 exposed to B[a]P over a period of 8 hours. Surprisingly, after 2 hours, a small and increasing amount of TACC3 begins to leave the nucleus of A2780 (Fig. 3). Intriguingly Ku70 also exits the nucleus. TACC3 is known to bind to and colocalize with Ku70 in the nucleus of A2780 (Lauffart et al

2007b). The PAH-induced effect appears specific as neither the arylhydrocarbon receptor, nor the histone acetyltransferase pCAF [also a TACC3 interacting protein (see Eslinger et al 2009 for review)] exits the nucleus. Ku70 is known to have a number of functions, of relevance to oncogenic transformation including transcriptional activation or repression of a subset of genes (Gullo et al 2006). Thus, export of Ku70 may also contribute to expression of the PAH-battery by removal of another potential transcriptional repressor from the nucleus. However, a relatively new role for Ku70 in the control of Bax-mediated apoptosis in the cytoplasm has been identified (Cohen et al 2004). We have previously observed that siRNA-mediated repression of TACC3 significantly increased the sensitivity of A2780 to a DNA damaging agent adriamycin, in an apparently p53-dependent manner (Lauffart et al 2007a). Furthermore, a physical interaction between normal TACC3 and normal p53 may be an important mediator for establishing cell cycle arrest, repair of DNA damage and/or apoptosis (Lauffart et al 2007a). Thus, it is intriguing to speculate that the export of TACC3 and Ku70 from the nucleus is also involved in the regulation of similar cell cycle arrest and apoptotic events triggered by PAH exposure.

Conclusions and Perspectives

Previously published material and the data presented in this manuscript suggest that human TACC3 acts at several key points in the response to

PAH and the DNA damage they cause (Fig. 4). This suggests that functional loss of TACC3 from the nucleus, noted in 100% of tumors analyzed to date, could increase aberrations in cell cycle control and DNA damage repair/responses when cells are exposed to PAH, contributing to the accumulation of mutations that results in the dedifferentiation and malignant development of the ovarian surface epithelium.
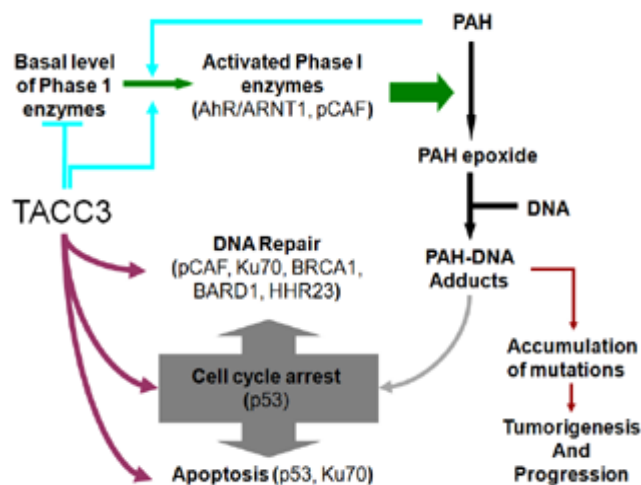

Figure 4: A functional model for TACC3's involvement in PAH-induced events in the OSE. TACC3 normally inhibits the basal expression of Phase 1 metabolizing enzymes by recruitment of histone deacetylases to the promoters of the respective genes (blue inhibitory pathway). Upon insult by PAH (blue arrow), TACC3 could participate in the attempt to remove PAHs from the system by recruiting AhR/ARNT to the promoters of genes encoding PAH metabolizing protein, and switching out HDACs for histone acetyltransferases, such as pCAF and CBP/p300 (blue arrow). This would increase the expression of the Phase 1 enzymes such as CYP1A1 (green arrow), converting PAH to the damaging epoxide. Accumulation of PAH-DNA adducts normally triggers cell cycle arrest and either correct DNA repair or apoptosis (grey arrow) Direct physical/functional interactions of TACC3 with cell cycle regulator p53, DNA damage response and repair complexes (containing BARD1/BRCA1, HHR23 Ku70 and pCAF) and apoptotic regulators (p53 and Ku70) could then promote restoration of normal cellular function by removing DNA adducts and mutations that they may induce, or death of the damaged cell. Functional loss of TACC3, early in the tumorigenic cycle, could increase the basal level of CYP1A1 in the OSE, increasing the turnover of PAH to the DNA-damaging epoxide and subsequent accumulation of PAH-DNA adducts. Absence of TACC3 from complexes involved in cell cycle arrest and DNA damage response/repair could also aid in accumulation of mutations either as an early or late event contributing to an increased risk of tumorigenesis and progression, (red arrows). Proteins involved in these pathways, which are known to interact directly or indirectly with TACC3, are indicated in parentheses.

Literature cited

Abe S and **M Sasaki**. 1982. SCE as an Index of mutagenesis and/or carcinogenesis. *In*: Sandberg AA, editor. Sister chromatid exchange. Progress and topics in cytogenetics. Alan R. Liss, Inc (New York). p461-514.

Angrisano T, **F Lembo**, **R Pero**, **F Natale**, **A Fusco**, **VE Avvedimento**, **CB Bruni** and **L Chiariotti**. 2006. TACC3 mediates the association of MBD2 with histone acetyltransferases and relieves transcriptional repression of methylated promoters. Nucleic Acids Research 34:364-372

Cohen HY, **S Lavu**, **KJ Bitterman**, **B Hekking**, **TA Imahiyerobo**, **C Miller**, **R Frye**, **H Ploegh**, **BM Kessler** and **DA Sinclair**. 2004. Acetylation of the C terminus of Ku70 by CBP and pCAF controls Bax-mediated apoptosis. Molecular Cell 13:627-38.

Delerive P, **Y Wu**, **TP Burris**, **WW Chin** and **CS Suen**. 2002. PGC-1 functions as a transcriptional coactivator for the retinoid X receptors. Journal of Biological Chemistry 277:3913-7.

Eslinger MR, **B Lauffart** and **IH Still**. 2009. TACC3 (transforming, acidic coiled-coil containing protein 3). Atlas of Genetics and Cytogenetics in Oncology and Haematology 14:305-310.

Forozan F, **EH Mahlamaki**, **O Monni**, **Y Chen**, **R Veldman**, **Y Jiang**, **GC Gooden**, **SP Ethier**, **A Kallioniemi** and **OP Kallioniemi**. 2000. Comparative genomic hybridization analysis of 38 breast cancer cell lines: a basis for interpreting complementary DNA microarray data. Cancer Research 60:4519-4525.

Gangisetty O, **B Lauffart**, **GV Sondarva**, **DM Chelsea** and **IH Still**. 2004. The transforming acidic coiled coil proteins interact with nuclear histone acetyltransferases. Oncogene 23:2559-63.

Garriga-Canut M and **SH Orkin**. 2004. Transforming acidic coiled-coil protein 3 (TACC3) controls friend of GATA-1 (FOG-1) subcellular localization and regulates the association between GATA-1 and FOG-1 during hematopoiesis. Journal of Biological Chemistry 279:23597-605.

Analysis of Human TACC3 in Cellular Responses to Polycyclic Aromatic Hydrocarbons

Gayther SA, **P Russell**, **P Harrington**, **AC Antoniou**, **DF Easton** and **BA Ponder**. 1999. The contribution of germline BRCA1 and BRCA2 mutations to familial ovarian cancer: no evidence for other ovarian cancer-susceptibility genes. American Journal of Human Genetics 65:1021-9.

Glockzin S, **FX Ogi**, **A Hengstermann**, **M Scheffner** and **C Blattner**. 2003. Involvement of the DNA repair protein hHR23 in p53 degradation. Molecular Cell Biology 23:8960-9.

Green A, **D Purdie**, **C Bain**, **V Siskind** and **PM Webb**. 2001. Cigarette smoking and risk of epithelial ovarian cancer (Australia). Cancer Causes and Control 12:713-9.

Greenlee RT, **T Murray**, **S Bolden** and **PA Wingo**. 2000. Cancer statistics, 2000. CA: a cancer journal for clinicians 50:7-33.

Gullo C, **M Au**, **G Feng** and **G Teoh**. 2006. The biology of Ku and its potential oncogenic role in cancer. Biochimica et Biophysica Acta. 1765: 223-234.

Harvey RG. 1991. Polycyclic Aromatic Hydrocarbons: Chemistry and Cancer. Cambridge University Press (Cambridge, UK).

Jakoby WB, **JR Bend** and **J Caldwell**. 1982. Metabolic Basis of Detoxification: Metabolism of Functional Groups. Academic Press (New York).

Jeffy BD, **RB Chirnomas**, **EJ Chen**, **JM Gudas** and **D Romagnolo**. 2002. Activation of the aromatic hydrocarbon receptor pathway is not sufficient for transcriptional repression of BRCA-1: requirements for metabolism of benzo[a]pyrene to 7r,8t-dihydroxy-9t,10-epoxy-7,8,9,10-tetrahydro benzo[a]pyrene. Cancer Research 62:113-21.

Jeffy BD, **EU Schultz**, **O Selmin**, **JM Gudas**, **GT Bowden** and **D Romagnolo**. 1999. Inhibition of BRCA-1 expression by benzo[a]pyrene and its diol epoxide. Molecular Carcinogenesis 26:100-18.

Jordan SJ, **DC Whiteman**, **DM Purdie**, **AC Green** and **PM Webb**. 2006. Does smoking increase risk of ovarian cancer? A systematic review. Gynecologic Oncology 103:1122-9.

Kao HY, **P Ordentlich**, **N Koyano-Nakagawa**, **Z Tang**, **M Downes**, **CR Kintner**, **RM Evans** and **T Kadesch**. 1998. A histone deacetylase corepressor complex regulates the Notch signal transduction pathway. Genes and Development 12:2269-77.

Langseth H and **K Kjaerheim**. 2004. Ovarian cancer and occupational exposure among pulp and paper employees in Norway. Scandinavian Journal of Work and Environmental Health 30:356-361.

Lauffart B, **O Gangisetty** and **IH Still**. 2007a. Evolutionary conserved interaction of TACC2/TACC3 with BARD1 and BRCA1: potential implications for DNA damage response in breast and ovarian cancer. Cancer Therapy 5:409-416.

Lauffart B, **G Sondarva**, **O Gangisetty**, **M Cincotta** and **IH Still**. 2007b. Interaction of TACC proteins with the FHL family: implications for ERK signaling. Journal of Cell Communication and Signaling 1:5-15.

Lauffart B, **MM Vaughan**, **R Eddy**, **D Chervinsky**, **RA DiCioccio**, **JD Black** and **IH Still**. 2005. Aberrations of TACC1 and TACC3 are associated with ovarian cancer. BMC Women's Health 5:8.

Leung YK, **KM Lau**, **J Mobley**, **Z Jiang** and **SM Ho**. 2005. Overexpression of cytochrome P450 1A1 and its novel spliced variant in ovarian cancer cells: alternative subcellular enzyme compartmentation may contribute to carcinogenesis. Cancer Research 65:3726-34.

Ma Q and **KT Baldwin**. 2000. 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced degradation of aryl hydrocarbon receptor (AhR) by the ubiquitin-proteasome pathway. Role of the transcription activation and DNA binding of AhR. Journal of Biological Chemistry 275:8432-8.

MacLeod MC, **G Adair**, **A Daylong**, **L Lewand** and **RM Humphrey**. 1991. Low absolute mutagenic efficiency but high cytotoxicity of a non-bay region diol epoxide derived from benzo[a]pyrene. Mutation Research 261:281-93.

McFadyen MC, **ME Cruickshank**, **ID Miller**, **HL McLeod**, **WT Melvin**, **NE Haites**, **D Parkin** and **GI Murray**. 2001. Cytochrome P450 CYP1B1 over-expression in primary and metastatic ovarian cancer. British Journal of Cancer 85:242-6.

Nagase S, **S Sato**, **F Tezuka**, **Y Wada**, **A Yajima** and **A Horii**. 1996. Deletion mapping on chromosome 10q25-q26 in human endometrial cancer. British Journal of Cancer 74: 1979-1983.

Ng JM, **W Vermeulen**, **GT van der Horst**, **S Bergink**, **K Sugasawa**, **H Vrieling** and **JH Hoeijmakers**. 2003. A novel regulation mechanism of DNA repair by damage-induced and RAD23-dependent stabilization of xeroderma pigmentosum group C protein. Genes and Development 17:1630-45.

Niwa Y, K Wakai, S Suzuki, K Tamakoshi, Y Lin, H Yatsuya, T Kondo, K Nishio, A Yamamoto, *et al*. 2005. Cigarette smoking and the risk of ovarian cancer in the Japanese population: findings from the Japanese Collaborate Cohort study. Journal of Obstetrics and Gynaecological Research 31:144-51.

Pan SY, AM Ugnat, Y Mao, SW Wen and KC Johnson. 2004. Association of cigarette smoking with the risk of ovarian cancer. International Journal of Cancer 111:124-30.

Ramus SJ, PD Pharoah, P Harrington, C Pye, B Werness, L Bobrow, A Ayhan, D Wells, A Fishman, *et al*. 2003. BRCA1/2 Mutation Status Influences Somatic Genetic Progression in Inherited and Sporadic Epithelial Ovarian Cancer Cases. Cancer Research 63:417-423.

Rossing MA, KL Cushing-Haugen, KG Wicklund and NS Weiss. 2008. Cigarette smoking and risk of epithelial ovarian cancer. Cancer Causes and Control 19:413-20.

Sadek CM, S Jalaguier, EP Feeney, M Aitola, AE Damdimopoulos, M Pelto-Huikko and J Gustafsson. 2000. Isolation and characterization of AINT: a novel ARNT interacting protein expressed during murine embryonic development. Mechanisms of Development 97:13-26

Sasaki MS. 1982. Sister chromatid exchange as reflection of cellular DNA repair. *In*: Sandberg AA, editor. Sister chromatid exchange. Progress and topics in cytogenetics.: Alan R. Liss, Inc. (New York). p135-61

Sato T, H Saito, R Morita, S Koi, JH Lee and Y Nakamura. 1991. Allelotype of human ovarian cancer. Cancer Research 51:5118-5122.

Schnekenburger M, L Peng and A Puga. 2007. HDAC1 bound to the Cyp1a1 promoter blocks histone acetylation associated with Ah receptor-mediated trans-activation. Biochimica et Biophysica Acta 1769:567-578

Schreiber E, P Matthias, MM Müller and W Schaffner. 1989. Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells. Nucleic Acids Research 17:6419

Sherwood JB, N Shivapurkar, WM Lin, R Ashfaq, DS Miller, AF Gazdar and CY Muller. 2000. Chromosome 4 deletions are frequent in invasive cervical cancer and differ between histologic variants. Gynecologic Oncology 79:90-96.

Suzuki S, DH Moore, DG Ginzinger, TE Godfrey, J Barclay, B Powell, D Pinkel, C Zaloudek, K Lu, *et al*. 2000. An approach to analysis of large-scale correlations between genome changes and clinical endpoints in ovarian cancer. Cancer Research 60:5382-5.

Tcherepanova I, P Puigserver, JD Norris, BM Spiegelman and DP McDonnell. 2000. Modulation of estrogen receptor-alpha transcriptional activity by the coactivator PGC-1. Journal of Biological Chemistry 275:16302-8.

Terry PD, AB Miller, JG Jones and TE Rohan. 2003. Cigarette smoking and the risk of invasive epithelial ovarian cancer in a prospective cohort study. European Journal of Cancer 39:1157-64.

Toyooka T, Y Ibuki, M Koike, N Ohashi, S Takahashi and R Goto. 2004. Coexposure to benzo[a]pyrene plus UVA induced DNA double strand breaks: visualization of Ku assembly in the nucleus having DNA lesions. Biochemical and Biophysical Research Communications 322:631-6.

Tsuchiya Y, M Nakajima and T Yokoi. 2003. Critical enhancer region to which AhR/ARNT and Sp1 bind in the human CYP1B1 gene. Journal of Biochemistry (Tokyo) 133:583-92.

Wagner SA, P Beli, BT Weinert, ML Nielsen, J Cox, M Mann and C Choudhary. 2011. A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles. Molecular and Cellular Proteomics 10:1-13.

Wang ZC, M Lin, LJ Wei, C Li, A Miron, G Lodeiro, L Harris, S Ramaswamy, DM Tanenbaum, *et al*. 2004. Loss of heterozygosity and its correlation with expression profiles in subclasses of invasive breast cancers. Cancer Research 64:64-71.

Werness BA, SJ Ramus, AS Whittemore, K Garlinghouse-Jones, I Oakley-Girvan, RA Dicioccio, Y Tsukada, BA Ponder and MS Piver. 2000. Histopathology of familial ovarian tumors in women from families with and without germline BRCA1 mutations. Human Pathology 31:1420-4.

Wu J, BB Zhu, J Yu, H Zhu, L Qiu, MS Kindy, L Gu, A Seidel and GM Li. 2003. In vitro and in vivo modulations of benzo[c]phenanthrene-DNA adducts by DNA mismatch repair system. Nucleic Acids Research 31:6428-34.

Yang LL, **VM Maher** and **JJ McCormick**. 1982. Relationship between excision repair and the cytotoxic and mutagenic effect of the 'anti' 7,8-diol-9,10-epoxide of benzo[a]pyrene in human cells. Mutation Research 94:435-47.

Zhang L, **U Savas**, **DL Alexander**, and **CR Jefcoate**. 1998. Characterization of the mouse Cyp1B1 gene. Identification of an enhancer region that directs aryl hydrocarbon receptor-mediated constitutive and induced expression. Journal of Biological Chemistry 273:5174-83.

Zhang Y, **PF Coogan**, **JR Palmer BL Strom** and **L Rosenberg**. 2004. Cigarette smoking and increased risk of mucinous epithelial ovarian cancer. American Journal of Epidemiology 159:133-9.