UNIVERSITY OF TARTU
Faculty of Science and Technology
Institute of Technology

Mykhailo Lytvynenko

Analysis of Aryl Hydrocarbon Receptor and p53 Expression in Various Pancreatic Cancer Cell Lines

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Supervisor(s):
Lilian Kadaja-Saarepuu, PhD
Indrek Teino, PhD
Pauliine Konsa, BSc

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Analysis of Aryl Hydrocarbon Receptor and p53 Expression in Various Pancreatic Cancer Cell Lines

Abstract:

Pancreatic adenocarcinoma is an extremely lethal disease. Analysis of underlying patterns can allow for development of more successful treatments. Aryl hydrocarbon receptor (AHR) has been shown to have an important role in cancer formation and progression. Interestingly, tumour suppressor p53 has been shown to have a correlation with the receptor in other cancers. Present work provides basis for future research into AHR and p53 relation not only in pancreatic adenocarcinomas, but other cancers as well.

Keywords:

Aryl hydrocarbon receptor, tumour suppressor p53, human pancreatic infiltrating ductal adenocarcinoma

CERCS: B220 Genetics, cytogenetics; B200 Cytology, oncology, carcinology.

Arüülsüsivesinike retseptori ja p53 ekspressiooni analüüs erinevates pankreasevähi rakuliinides

Lühikokkuvõte:

Inimese pankrease adenokartsinoomi loetakse üheks kõige letaalsemaks vähitüübiks. Seetõttu on oluline uurida antud vähi tekke ja arengumehhansime, et võimaldada tulevikus uute ravimite välja töötamist. Varasemalt on näidatud, et arüülsüsivesinike retseptor (AHR) mängib olulist rolli nii kasvajate tekkes kui arengus. Lisaks sellele on eri vähitüüpides seostatud AHR-i tuumor supressoriga p53. Käesolev töö on aluseks AHR-i ja p53 seoste uurimisele nii pankrease adenokartsinoomis kui ka teistes kasvajates.

Võtmesõnad:

Arüülsüsivesinike retseptor, tumor suppressor p53, inimese infiltreeruv pankreasejuha adenokartsinoom

CERCS: B220 Geneetika, tsütogeneetika; B200 Tsütoloogia, onkoloogia, kantseroloogia.

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TERMS, ABBREVIATIONS AND NOTATIONS

AHR Aryl hydrocarbon receptor

AHRE Aryl hydrocarbon receptor response element

AHRR Aryl hydrocarbon receptor repressor

AIP AHR-interacting protein

ARF Alternative reading frame

ARNT Aryl hydrocarbon receptor nuclear translocator

ATM Serine-protein kinase

ATR Serine/threonine-protein kinase

bHLH basic Helix-Loop-Helix protein domain

bHLH-PAS basic Helix-Loop-Helix PER/ARNT/SIM protein family

CYP1A1 Cytochrome P450 family 1 subfamily A member 1

CYP1B1 Cytochrome P450 family 1 subfamily B member 1

DBD DNA-binding domain

HAH Halogenated aromatic hydrocarbons

HSP90 Heat shock protein 90

ID Inhibitory domain

MDM2 Mouse double minute 2 homologue protein

NES Nuclear export sequence

NLS Nuclear localization sequence

OD Oligomerization domain

PAH Polycyclic aromatic hydrocarbons

PAS PER/ARNT/SIM domain

PIDA Pancreatic Infiltrating Ductal Adenocarcinoma

PR Proline-rich domain

TAD Transactivation domain

INTRODUCTION

Pancreatic cancer is a debilitating disease with comparatively low incidence, yet high mortality rates. Most common cases are pancreatic adenocarcinomas. Risk factors include but not limited to tobacco smoking, diabetes type 1 and 2, alcohol consumption and obesity. Unfortunately, early stages of this disease tend to be asymptomatic, and cancer can proliferate discreetly, often to late stages of a metastatic carcinoma.

Discovery of underlying progression mechanisms allows for treatments to target them and challenge the disease. Therefore, understanding and analysing regulatory and receptor genes' link to cancer has been the goal of researchers for decades. Cellular recognition of protumourigenic carcinogen process plays an important role in organism's senescence. Among them are different environmental pollutants. Many of them are aryl hydrocarbon receptor (AHR) ligands. Consequently, AHR, depending on the ligand, activates according target genes. Yet, it is not the only role the receptor plays, as its role can be seen in facilitating signals in homeostasis, such as its role in ovarian granulosa cells. AHR's control over cells growth, maturation and controlled death can be an important cancer target.

Other important proteins for controlling the cells proliferation are tumour suppressors. Perhaps best known and most aggressively studied is tumour suppressor protein p53, an important tool in protecting cells from cancer. It is a DNA-binding protein and regulates target genes, which mediate cellular response from stress signals to important cell fate decisions of cell cycle arrest, apoptosis or DNA repair.

The aim of the present work was to analyse both AHR and p53 expression on mRNA and protein levels in pancreatic infiltrating ductal adenocarcinoma cell lines.

1 LITERATURE REVIEW

1.1 Pancreas

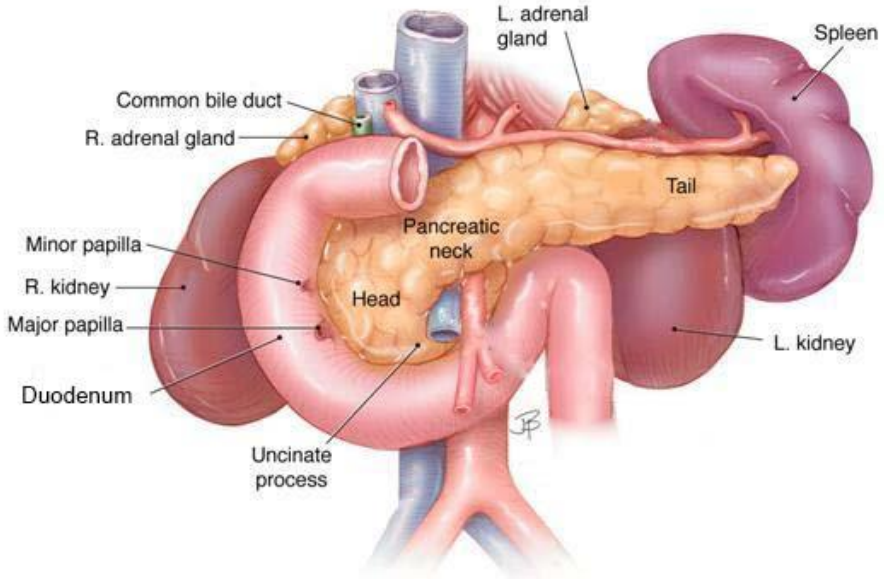

Figure 1. Anatomy of the pancreas and its relationship to the surrounding structures and organs. Image taken and modified from "Anatomy and Histology of the Pancreas" (Longnecker, 2021).

Pancreas, as defined by National Cancer Institute, is glandular organ located in the abdomen (Cancer.gov). Its functions can be divided into exocrine and endocrine. Exocrine function is responsible for secretion of digestive enzymes, commonly referred to as pancreatic juice, into the duodenum and is performed by 95% of the pancreatic mass. Endocrine function is responsible for the production and secretion of enzymes into the bloodstream, thereby controlling energy metabolism and storage. Prime example of pancreatic enzymes would be insulin and glucagon. Endocrine pancreas is comprised out of islets, which constitute 1-2% of pancreatic mass. The shape of the organ is irregularly prismatic (Fig.1). The Pancreas's right-most extremity, situated within the curve of the duodenum, is the head. The head is, consequently, a region of attachment of pancreatic and common bile ducts to the duodenum. Pancreatic duct extends transversely from left to right extremities and supplies pancreatic juice through major papilla to the small intestines. The extension of the head, bending backwards, is called the uncinate process. Main portion of the pancreas is called the body. Head and body of the pancreas are connected by a slight constriction, called the neck. The left-most extremity of the pancreas is called the tail.

1.2 Pancreatic Cancer

According to American Cancer Society, the average lifetime risk of pancreatic cancer is approximately 1 in 64 (American Cancer Society, 2022). It has one of the lowest survival rates out of all cancer types. The combined 5-year survival rate is estimated 11%, according to the National Cancer Institute's database "Surveillance, Epidemiology, and End Results". (American Cancer Society, 2022) One reason for this is that the early stages of pancreatic cancer are seldomly diagnosed. Early diagnose, however, is important as this results in a more successful treatment and recovery of patients. The initial stages are frequently asymptomatic, with symptoms appearing in metastatic stage of cancer, as the tumour spreads to other organs. With this in consideration, projections predict that pancreatic cancer will become second cancer by lethal outcomes by 2030, second only to lung (Rahib et al., 2014).

The prevailing number of pancreatic cancers are classified as pancreatic adenocarcinoma. It is responsible for 95% of pancreatic cancer cases (Clevelandclinic.org). Adenocarcinoma is the development of a malignant tumour in cell lining of glandular organ (glandular epithelial cells). The most common in pancreas is infiltrating ductal adenocarcinoma (PIDA). Majority of PIDA cases arise within the head of the pancreas and less commonly in body or tail. The cells of PIDA are disorganized and adjacent to histological structures, such as vessels, without intervening intermediate structures: stroma or acini. Generally, ductal adenocarcinomas can be characterized by significantly enlarged nuclear region of the cells and high frequency of mitotic divisions. (Sanchez & Cheung, 2015)

1.3 Aryl hydrocarbon receptor

Aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor, discovered as an intermediary to an environmental pollutant 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (Poland et al., 1976). After the original publication on AHR's role in aryl hydrocarbon hydroxylase (CYP1A1 – cytochrome P450 family 1 subfamily A member 1) activation by 24 different polycyclic hydrocarbons, many publications and works have continued to clarify and expound the toxicological significance of the receptor. CYP1A1 belongs to a cytochrome P450 family of enzymes, along with CYP1B1 (cytochrome P450 family 1 subfamily B member 1) and both are direct AHR target genes. The functions of CYP1A1 include degradation of xenobiotic agonists, while CYP1B1 engages in endogenous metabolism of fatty acids, steroid hormones and vitamins (Badawi et al., 2001). AHR, although discovered as a

xenobiotic receptor, would continually have additional roles be investigated. Proceeding research has provided an endogenous role for AHR and show the effect and role of the transcription factor in various diseases and health conditions.

DNA microchip analysis has shown that the receptor has direct and indirect effect on transportation pathways, lipid and cholesterol synthesis, as well as energy metabolism (Sato, 2008). AHR has exhibited functional importance in development and maturation of embryonic and hematopoietic stem/progenitor cells (Ko et al., 2016; Latchney et al., 2011; Mallavarapu et al., 1999). Latest research has presented inconsistent evidence of both protumourigenic and antitumourigenic effects of AHR. Immunohistochemical analysis of tumours from breast, prostate, gastric, small cell lung, renal and liver cancers show increase in AHR expression levels in comparison to surrounding tissue (Ishida et al., 2015; Liu et al., 2013; Powell et al., 2013; Richmond et al., 2014; Saito et al., 2014; Su et al., 2013; Yin et al., 2013). These findings have instigated research into AHR as a cancer treatment target (Safe et al., 2013).

1.3.1 Aryl hydrocarbon receptor structure

Human gene of *AHR* is located on the seventh chromosome. Gene name aliases include *BHLHE76* as a hint to the family of proteins it belongs to — the basic helix-loop-helix PER/ARNT/SIM (bHLH-PAS) protein family.

AHR gene consists out of 11 exons, translated to 848 amino acid long protein from the 6234 bp mRNA template. Molecular weight of the protein is approximately 96 kDa (96.147 kDa). (Uniprot.org; Ensembl.org A)

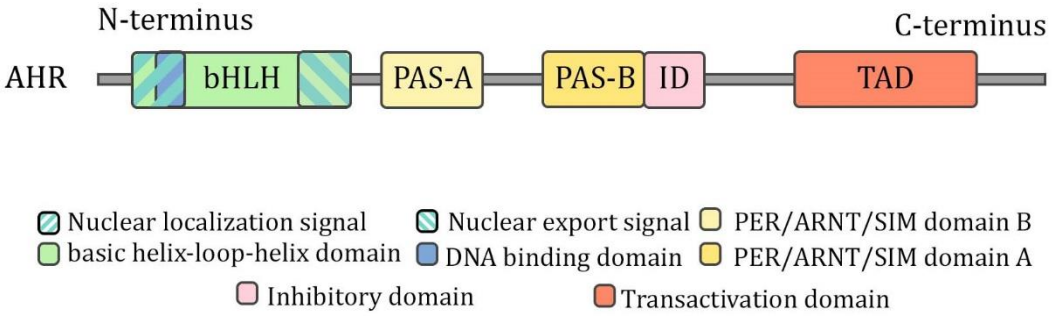

Figure 2. Schematic representation of AHR domain structure. Image modified from I. Teino PhD thesis "*Studies on aryl hydrocarbon receptor in the mouse granulosa cell model"*. (Teino Indrek, 2020)

The key regions in AHR signalling, nuclear localization and nuclear export sequences (NLS and NES, respectively), are located in the N-terminal region of the protein (Fig.2) (Ikuta et al., 1998). With a certain overlap, bHLH has basic amino acids are responsible for AHR binding to DNA and a HLH motif accountable for aryl hydrocarbon receptor nuclear translocator (ARNT)-AHR heterodimerization (Schulte et al., 2017). Following domain, PAS, has also been identified in proteins PER, ARNT and SIM, from which it received its name. In AHR, PAS is present in two inverted repeats ─ PAS-A and PAS-B. Former of them warrants AHR heterodimerization with the aforementioned ARNT protein (Wu et al., 2013). PAS-A and PAS-B can each bind a heat shock protein 90 (HSP90), but only latter has other cytoplasmic targets. They include AHR-interacting protein (AIP) and p23, a HSP90 co-chaperone protein (Bell & Poland, 2000; Kazlauskas et al., 1999; Perdew, 1988). Inhibitory domain (ID) of AHR is connected with PAS-B. Functionally, ID provides inhibition of transactivating capabilities of the protein (Ma et al., 1995). The C-terminal region is highly variable, with a disordered region at the tailing part of the sequence. However preceding it, is a glutamine rich (Q-Rich) region of a transactivation domain (TAD), that provides transactivating functions on AHR's target genes and allows for binding of such cofactors, as histone acetyl transferase p300 (EP300), receptor-interacting protein 140 (RIP140), steroid receptor coactivator 1 (SRC-1) and silencing mediator of retinoic acid and thyroid hormone receptors (SMRT) (Kumar et al., 1999; Kumar & Perdew, 1999; Rushing & Denison, 2002; Tohkin et al., 2000).

1.3.2 Signalling and regulation of aryl hydrocarbon receptor

The earliest discovered and most common pathway for AHR is termed the genomic pathway (Fig.3). In its most elementary understanding, proceeding activation, AHR translocates to the nucleus, forms a heterodimer with ARNT and binds the AHR response element (AHRE). AHRE has been alternatively termed as a xenobiotic or dioxin response elements (XRE/DRE). Among AHR/ARNT target genes are for example CYP1A1, CYP1B1, CYP1A2, aryl hydrocarbon receptor repressor (AHRR) and apoptosis regulator BAX (Baba et al., 2001; Black & Quattrochi, 2004; Eltom et al., 1999; Matikainen et al., 2001; Watson & Hankinson, 1992). In an inactivated state, the resting AHR resides in the cytoplasm and is stabilised by AIP, p23 and a HSP90dimer. Upon binding an agonist, AHR undergoes a confirmational change. Proceedingly, exposed NLS domain will be recognized by importin ß and AHR translocated into the nucleus of the cell (Petrulis et al., 2003). Inside the nucleus,

liganded AHR sheds chaperoning proteins, binds ARNT and consequently activates AHR DNA binding domain (Heid et al., 2000; Lees & Whitelaw, 1999; McGuire et al., 1994; Soshilov & Denison, 2008). Classical binding sequence for the heterodimer reads 5'-TNGCGTG-3' (Denison et al., 1988). Half-site 5'-TNGC-3' is AHR recognized, leaving ARNT to target 5'-GTG-3'. Flanking nucleotides of the consensus region, likewise, play an important role. Downstream AHRE positions +1 and +3 with nucleotides A and T have been shown as crucial for gene transactivation (Matikainen et al., 2001). Subsequently, AHR/ARNT complex may request cofactors to modulate gene expression. Eventually, AHR/ARNT complex dissociates. AHR is ubiquitinated, translocated to the cytoplasm and degraded by the 26S proteasome pathway (Ma & Baldwin, 2000).

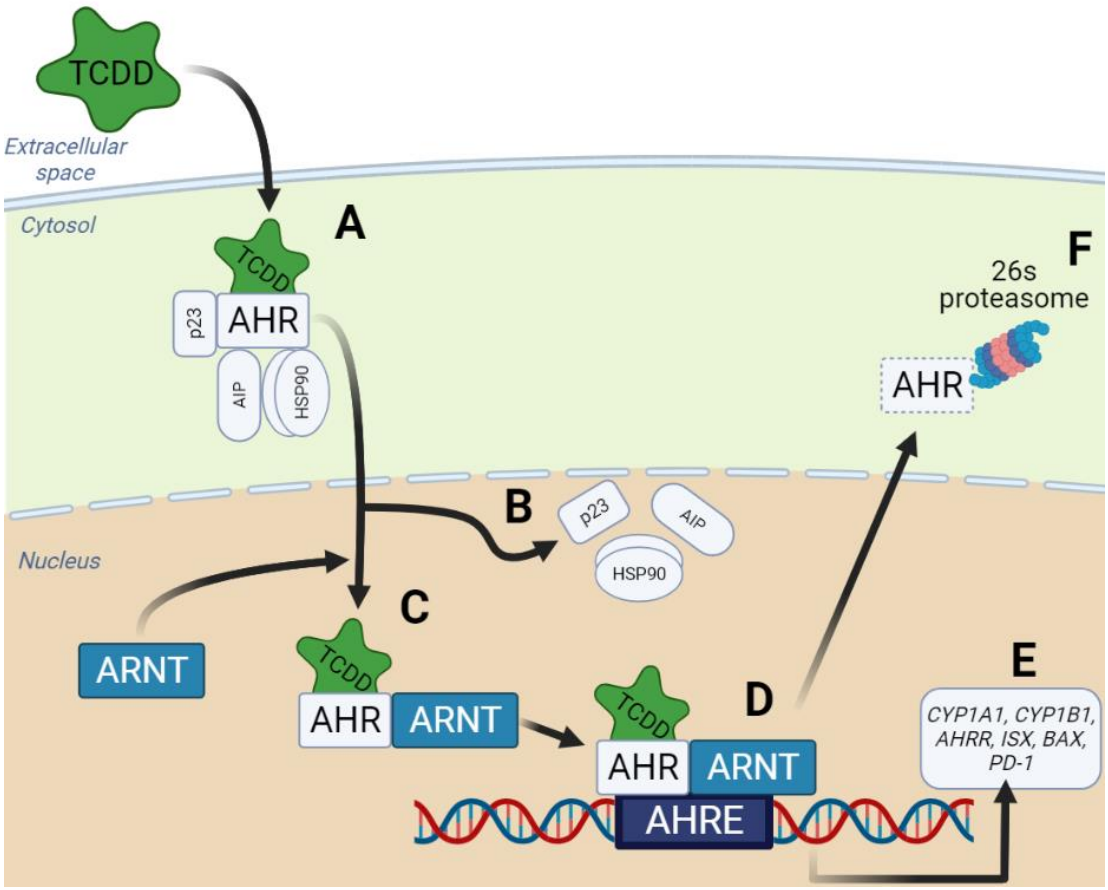

Figure 3. Genomic signalling pathway of AHR. (A) Exogenous ligand (e.g., TCDD) binds to the AHR. Receptor is in its stabilising complex consisting of p23, AIP and two units of HSP90. Upon ligand binding, AHR exposes its NLS and translocates to the nucleus. (B) When AHR enters the nucleus, it sheds its chaperone proteins and (C) binds ARNT. (D) AHR-ARNT complex then recognizes and attaches to AHRE and (E) influences their transcription. (F) Complex dissociates, AHR is exported to the cytoplasm, where it is degraded by the 26s proteasome. Image modified from I. Teino PhD thesis "*Studies on aryl hydrocarbon receptor in the mouse granulosa cell model"*. (Teino Indrek, 2020)

Regardless, AHR role is not limited to its genomic pathway. Non-genomic pathway has also been outlined. Recent studies present evidence of its importance in immune response in human monocyte derived macrophages (Großkopf et al., 2021). However, the current work will concentrate on the genomic pathway, as it is almost directly controlled by AHR/ARNT complex and allows for straightforward analysis with CYP1A1, as its single activator is AHR.

1.3.3 Aryl hydrocarbon receptor in cancer

To have a better evaluation of AHR in tumourigenesis, a deeper look into receptor's activators (agonists) is necessary. The ligands can be divided into two classes of chemicals: anthropogenic agonists and natural agonists.

Anthropogenic agonists can be considered the initial ligand group, as originally only environmental contaminants were believed to be sole interactors. These include halogenated aromatic hydrocarbons (HAHs) and polycyclic aromatic hydrocarbons (PAHs). Such contaminants are often generated in reactions of combustion or as side products in chemical industry. HAHs group includes polychlorinated dibenzo-p-dioxins (e.g., high affinity AHR ligand TCDD), dibenzofurans (e.g., 2,3,7,8-tetrachlorodibenzofuran) and biphenyls (e.g., PCB126) (Farrell et al., 1987; J. M. Jones & Anderson, 1999; Poland et al., 1976). PAHs are comprised from chemicals containing minimally four benzene rings, including benzo(a)pyrene and 3-methylcholanthrene (J. M. Jones & Anderson, 1999; Piskorska-Pliszczynska et al., 1986). Recent studies on effect of drugs contribute to the anthropogenic agonists. Anti-allergy drug Tranilast, neuroimmunomodulator Laquinimod, anti-malarial Primaquine have all been shown to activate AHR (Backlund & Ingelman-Sundberg, 2004; Hu et al., 2013; Kaye et al., 2016).

Natural agonists include popular industrial dyes that can also be found in humans naturally — indigo and indirubin (Adachi et al., 2001). Prostaglandins have also been identified as agonists, including heme metabolites bilirubin and biliverdin (Seidel et al., 2001). Latest research focus is beset on tryptophan metabolites. Ultraviolet (UV) generated 6-formylindolo(3,2-b)carbazole (FICZ) was depicted to have high affinity to AHR (Fritsche et al., 2007). A tryptophan-derived kynurenine (Kyn) that has recently gained interest for its role in tumour promotion and immune response escape, is also an AHR agonist (Mezrich et al., 2010; Opitz et al., 2011).

Recalling UV induced FICZ synthesis, AHR is connected to skin cancer. FICZ is capable of activating AHR and induce CYP1A1/1B1 target genes (Katiyar et al., 2000). Moreover, AHR can downregulate cell cycle inhibitor p27 and allow UV damaged cells survive and linger (Frauenstein et al., 2013). Lastly, activation of AHR represses cell's DNA repair systems (Pollet et al., 2018). But UV is not the only cancer promoting element, as multiple airborne carcinogens, including PAHs, have been identified (Gualtieri et al., 2011; Matsumoto et al., 2007).

Another prevalent cancer type, breast carcinoma, has shown importance of AHR in its progression. Earlier research found breast cancer type 1 susceptibility protein (BRCA1) often to be mutated or downregulated (Wilson et al., 1999). In following years, evidence of AHR positively regulating BRCA1 has been presented, while xenobiotics were absent (Hockings et al., 2006). In addition to this, in the same study, TCDD and benzo(a)pyrene have demonstrated abrogation of BRCA1 coactivators, driving regulation downwards. Notably, same regulation was shown with antagonist ligation to the AHR, providing evidence for a varied ligand-dependent interaction. Another study has presented AHR and tumour suppressor p53 negative correlation (Z. D. Li et al., 2014). Furthermore, TCDD can interfere with the phosphorylation and acetylation activity of p53 (Ambolet-Camoit et al., 2010).

AHR has also demonstrated its important signalling functions in pancreatic carcinomas. Prominent PIDA cell lines have been used to show AHR upregulation when compared to pancreatitis and normal pancreas (Koliopanos et al., 2002). However, some pancreatic cell lines would provide faint signals in northern blot analysis. Mediated by AHR, metastasis associated lung adenocarcinoma transcript 1 (MALAT1) is upregulated (Lee et al., 2020). MALAT1 would be also correlated to increasingly aggressive cases of pancreatic cancer (L. Li et al., 2016; Pang et al., 2015). Curiously, different adenocarcinomas, depending on their invasiveness, show once again AHR's ligand-dependent variation in functionality (Jin et al., 2015). Therefore, explication of AHR mechanisms in human PIDA cells is required, yet difficult to link cohesively in a unified and comprehensive manner.

1.4 Tumour suppressor p53

The easiest explanation for the role and effect of the tumour suppressor protein p53, encoded by *TP53* gene, in cellular homeostasis can be described by its unofficial title as the "Guardian of the Genome". p53 protein performs sensory and anti-oncogenic functions in response to

oncogenic, cytotoxic and ischemic stress signals. p53 was discovered in 1979 in parallel by six research groups (Deleo et al., 1979; Kress et al., 1979; Lane & Crawford, 1979; Linzer & Levine, 1979; Melero et al., 1979; Smith et al., 1979). Found both in human and mouse cells, it was initially envisioned as an oncogene, meaning a mutated form of a gene, capable of promoting cancer (Eliyahu et al., 1984, 1985; Jenkins et al., 1984; Parada et al., 1984). One of the main causes for this misconception is the high expressions of mutant p53 in carcinomas, yet undetectable level of wild type p53 in healthy cells.

However, in 1989 publication by Suzanne J. Baker and her colleagues presented evidence of p53 function as a tumour suppressor after performing a "two-hit" test (Baker et al., 1989; Knudson, 1971). Baker *et al.* clarified the functionality of *TP53* but also emboldened following researchers to apply the "two-hit" test to other tumour types. Afterwards, p53 was found to be frequently mutated in carcinomas (Nigro et al., 1989). In following years and subsequently, decades, *TP53* remained an important cancer therapy target, with evidence of *TP53* mutations actively promoting tumourigenesis (Rivlin et al., 2011).

1.4.1 Structure of tumour suppressor p53

TP53 is a gene located on the seventeenth human chromosome, measuring in approximately 20kb and consisting of 11 exons (Benchimol et al., 1985; Bienz et al., 1984; Lamb & Crawford, 1986). The length of human *TP53* mRNA transcript being 2.8 kb long and is translated to a 393 amino acid long protein with the molecular weight of 53 kDa. (Ensembl.org B;)

p53 protein family includes its homologs p63 and p73. *TP53* diverged from its family members to attain tumour suppressor capabilities, while remaining members exhibit embryogenic functions (Belyi et al., 2010). Family has multiple alternative splicing variants (Marshall et al., 2021). p63 and p73 have considerably larger count of isoforms. However, p53 has been shown to have N-terminally truncated form (Δ133 p53), courtesy of the alternative internal promoter region within intron 4 of the gene (Bourdon et al., 2005). Such isoform possessed dominant-negative effects on wild-type p53.

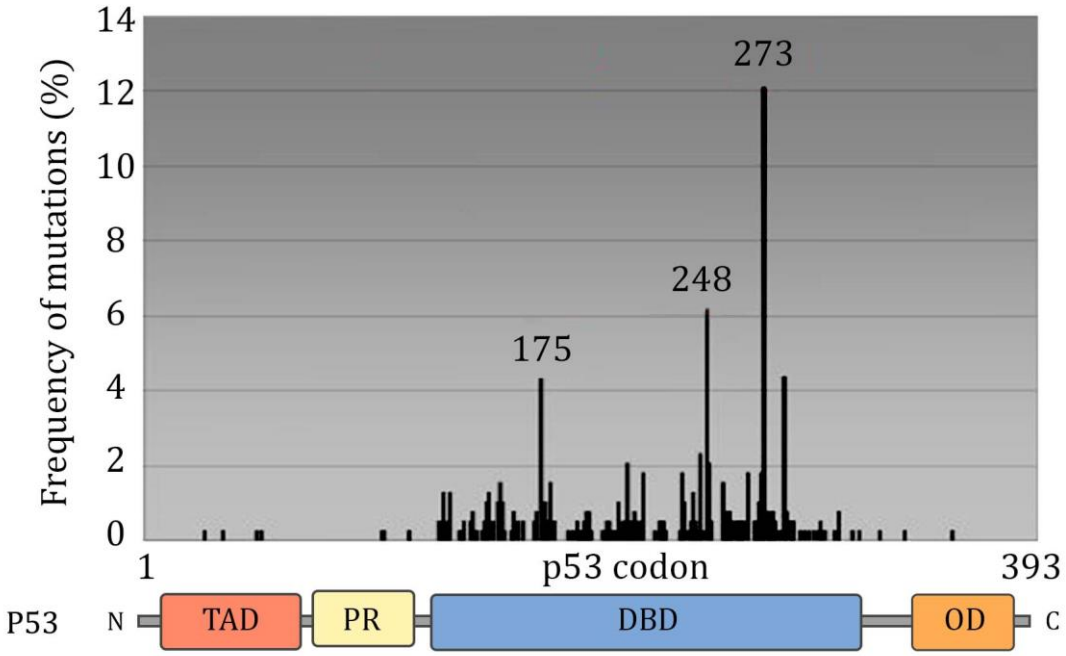

Figure 4. Schematic representation of p53 domain structure, frequency and location of its mutations in pancreatic cancer. TAD – transactivation domain; PR – Proline-rich region; DBD – DNA binding domain; OD – oligomerization domain. Images were taken and modified from Abhinav Jain, Michelle C. Barton (Jain & Barton, 2018) and UMD *TP53* Mutation Database (p53.free.fr).

Protein structure of p53 (Fig.4) has an N-terminal 42 amino sequence of the transactivation domain (TAD) (Lin et al., 1994). Following TAD is a proline-rich region (PR) and highly conserved DNA binding region (DBD). PR functions during activation of transcription and restriction of cell growth (Walker & Levine, 1996). DBD is a protease-resistant core and has a length of 200 amino acids. It is able to recognize a 10-base pair pattern of PurPur-PurCA/T.A./TPyrPyrPyr (Purine = Pur, Pyrymidine = Pyr) of p53 DNA-binding sites (Bargonetti et al., 1993; El-Deiry et al., 1992; Pavletich et al., 1993). Most cancer-associated mutations, rendering p53 unable to bind DNA and regulate gene expression, occur in this region (Fig.4). The oligomerization domain (OD) is located in the C-terminal and plays a crucial role in formation of a p53 tetramer and its subunits of two dimers (Jeffrey et al., 1995). Tetramerization is believed to play a vital role in cell fate decisions mediated by p53 (Fischer et al., 2016). OD region also contains an NES sequence, which gets masked in complete oligomerized units (Stommel et al., 1999). This function allows tetramers to be contained within the nucleus, while monomers and dimers get exported into the cytoplasm of the cell.

1.4.2 Signalling pathway role of p53

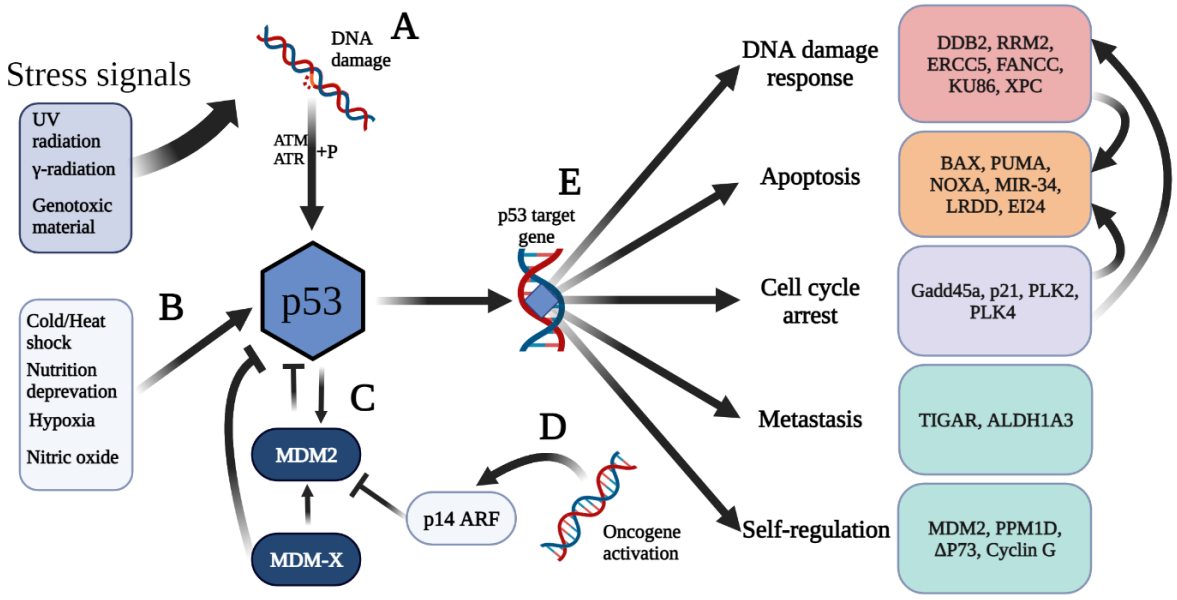

Figure 5. p53 response mechanism to endo- and exogenous stress signals. (A) DNA damage is induced by extracellular stress signals. p53 is activated by ATM and ATR phosphorylating the tumour suppressor (+P). (B) Extra- and intracellular stress signals can directly activate p53. (C) Negative feedback loop of p53 and its repressor MDM2. MDM2 can be induced by MDM-X, which is also inhibitory to p53. (D) p14 ARF responds to oncogene activation and represses MDM2, allowing p53 protein to be upregulated. (E) Genomic binding of a p53 tetramer and subsequent activation of five classes of targets. Classes are characterized by their cell-fate decision. Picture was taken and modified from Christine M. Eischen (Eischen, 2016).

The response to varying sources of stress by p53 results in p53-mediated activation of key genes leading to three predominant cell fate choices of apoptosis, cell cycle arrest or cell senescence (Fig.5) (Engeland, 2017; Mijit et al., 2020; Ozaki & Nakagawara, 2011). Cellular stress capable of activating p53 are DNA damage, hypoxia, nitric oxide or oncogenic activation. DNA damage cascade involves serine and serine/threonine-protein kinases (ATM and ATR, respectively) that phosphorylate p53 (Dai & Gu, 2010; G. G. Jones et al., 2004). Mechanism for oncogenic response occurs by p14 tumour suppressor alternative reading frame (ARF) protein repressing the main repressor of p53, a mouse double minute 2 homologue protein (MDM2) (Levine, 1997; Zhang et al., 1998).

Following p53 activation leads to transactivation of multiple groups of genes. These can be divided in 5 groups: (i) DNA damage response (e.g. damage specific DNA-binding protein 2 (*DDB2*) and ribonucleoside-diphosphate reductase subunit M2 (*RRM2*)); (ii) apoptotic cell fate (e.g. BCL-2-associated X, Apoptosis regulator (*BAX*) and p53 upregulated modulator of apoptosis (*PUMA*)); (iii) cell cycle arrest (e.g. cyclin-dependent kinase inhibitor1

(*CDKN1A*), translated into p21, and growth arrest and DNA-damage inducible alpha (*GADD45A*)); (iv) metabolism (e.g. *TP53*-induced glycolysis regulatory phosphatase (*TIGAR*) and aldehyde dehydrogenase one family, member A3 (*ALDH1A3*)); (v) p53 self-regulation (e.g. *MDM2* and protein phosphatase, Mg2+/Mn2+ dependent 1D (*PPM1D*)) genes (Hafner et al., 2019).

1.4.3 p53 in cancer

Genomic research into 12 tumour types with aims of analysing average prevalence of mutated *TP53*, showed that across all samples 42% of the cases had the altered sequence of the gene (Kandoth et al., 2013). However, frequency of the mutation is increased in most-persistent carcinomas, including lung, triple-negative breast, serous ovarian and esophageal cancers (D. Bell et al., 2011; Kandoth et al., 2013; Koboldt et al., 2012; Peifer et al., 2012; Song et al., 2014). Autosomal dominant germline mutation in *TP53* has been shown to cause the Li-Fraumeni syndrome (F. P. Li & Fraumeni, 1969). It is a rare cancer predisposition syndrome with an increased risk of multiple primary tumours progressing at the same time period (McBride et al., 2014; Ruijs et al., 2010).

2 THE AIMS OF THE THESIS

In order to uncover the correlation of AHR and p53 in pancreatic invasive ductal adenocarcinoma, the following aims were set:

- To analyze *AHR* expression and its pathway activity in PIDA cell lines.
- To analyze *TP53* expression in PIDA cell lines.
- To assess the relative protein abundance of AHR and p53 in select cell lines.

3 EXPERIMENTAL PART

3.1 MATERIALS AND METHODS

3.1.1 Cell lines and cell cultivation

In the experiments, PIDA cell lines AsPc1-WT (wild-type), AsPc1-KO (AHR-deficient), BxPc-3, Su86.86, and Capan-1 were used, which were acquired from our project partners from University of Oslo and Lithuanian University of Health Sciences. Cells were grown in RPMI 1640X media (Corning Inc.) containing 10% fetal bovine serum (FBS) (Capricorn Scientific) and 1% penicillin-streptomycin (Corning Inc.) on 100mm and 6-well plates (Greiner Bio-One). Cells were passaged with trypsin (Corning Inc.) every 3-5 days, depending on the growth rate, and incubated in a cell culture incubator at 37 °C and 5% CO_2.

3.1.2 RNA extraction

For cell lysis, the cells were washed once with PBS, followed by addition of FARB lysis buffer (Favorgen Biotech Corporation) containing 1% β-mercaptoethanol (Alpha Medthrift Scientific Co.). Lysates were collected, followed by RNA extraction with Tissue Total RNA Purification Mini kit (Favorgen Biotech Corporation) according to the manufacturer's protocol. RNA was eluted in 30 µl of nuclease-free water. Concentration was measured, and quality analysed with NanoDrop ND-100 (Thermo Fisher Scientific). RNAs were stored at -80 °C.

3.1.3 Reverse Transcription (RT)

First, DNase I treatment was performed in 10 µl containing 1000 ng of RNA, 1 X DNase I buffer, 10 U RiboLock, and 1 U DNase I (Thermo Fisher Scientific). Samples were incubated at 37 °C for 30 minutes, followed by reaction inactivation with 4,5 mM EDTA for 10 min at 65 °C. Reverse transcription was performed using RevertAid RT kit (Thermo Fisher Scientific) in 20 µl containing 1 X reaction buffer, 20 U RiboLock, 5 µM random hexamer primer, 1 mM dNTP, and 200 U revertase. The reaction was activated at 25 °C for 10 min, followed by cDNA synthesis at 42 °C for 60 min and inactivation at 70 °C for 10 min in 2720 Thermal Cycler (Applied Biosystems).

3.1.4 Quantitative polymerase chain reaction (qPCR)

For gene expression analysis, qPCR was performed on 384-well plates (Bioplastics) using LightCycler® 480 II (Roche) machine. The reaction mix (10 µl) contained 500nM forward and reverse primers, 5 µl SYBR™ Green/ROX qPCR Master Mix, and 4 µl cDNA. Water was used instead of cDNA as negative controls. Reactions were performed as triplicates, and the average Ct (cycle threshold) values, calculated with LightCycler 480 II program, were used in the analysis. Target gene expression was normalised with the house-keeping gene TBP (TATA-binding protein). Specificity of PCR products was determined by melting curve analysis. For relative gene expression calculations, the Livak method was used (Livak and Schmittingen, 2001). The qPCR program was as follows: initial denaturation at 95 °C for 10 min; 40 cycles of denaturation at 95 °C for 15 sec and primer annealing/elongation at 60 °C for 1 min; melting curve data was generated by a gradual increase of temperature from 45 °C to 95 °C over the time course of 7 min.

Primer sequences used:

AHR_F 5'-ATTACAGGCTCTGAATGGCTTTG-3'

AHR_R 5'-TGACATCAGACTGCTGAAACCCTAG-3'

CYP1A1_F 5'-GCTGACTTCATCCCTATTCTTCG-3'

CYP1A1_R 5'-TTTTGTAGTGCTCCTTGACCATCT-3'

TBP_F 5'-TGCACAGGAGCCAAGAGTGAA-3'

TBP_R 5'-CACATCACAGCTCCCCACCA-3'

p53_F 5'-CCTCAGCATCTTATCCGAGTGG-3'

p53_R 5'-TGGATGGTGGTACAGTCAGAGC-3'

3.1.5 SDS-PAGE and Western blot analysis

For lysis, cells were gathered in PBS with a cell scraper and centrifuged for 5 min at 300 rcf room temperature. For resuspension 50 µl of RIPA buffer (Radioimmunoprecipitation assay) (50 mM Tris pH 7,4, 1% Triton X-100, 1 mM EDTA, 150 mM NaCl, 0,1% SDS) and 1 x proteinase inhibitor (Roche) was used. Cells were placed on ice for 1 h and suspended after every15 min. Lysates were then centrifuged for 10 min at 4 °C 18 000 rcf; the supernatant was used for analysis or stored at -80 °C. Protein concentration was measured with Pierce

(TM) BCA protein assay kit (Thermo Fisher Scientific) using Multiscan Ascent (Thermo Fisher Scientific) system.

To prepare lysates for analysis, 1 x loading, 100 mM dithiothreitol, and RIPA were added to 20 µl of sample for a volume of 25 µl. The samples were then heated for 5 min at 95 °C. 25 µl of samples was loaded to 10 % SDS-polyacrylamide gel and separated by size using Mini-PROTEAN® Tetra Cell machine (Bio-Rad) for 45 min at 50 V and then at 170 V for 1 h. Polyvinylidene difluoride (PVDF) membrane was activated in methanol. After which, the membrane and gel were kept in the transfer buffer (192 mM glycine, 0,1% SDS, 25 mM Tris-HCl ph 8,3, 10% methanol) for 5 min. Using Trans-Blot® SD Semi-dry Transfer Cell (Bio-Rad) at 17 V for 15 min, the proteins were transferred from gel onto the PVDF membrane. Then the membrane was washed in TBST buffer (Tris-buffer, 0,5% Tween-20), and unspecific signals were blocked using blocking buffer (5 % non-fat milk in TBST) for 1 h. Incubation with primary antibody in blocking buffer was done overnight at 4 °C. The membrane was washed with TBST for 4 x 6 min before incubation with secondary antibody in blocking buffer for 1 h. After the incubation, 4 x 6 min washes with TBST were done, and for imaging, the membrane was covered with Immobilon Western 21 Chemiluminescent HRP substrate mix (Merck Millipore) for 5 min. Images were obtained using CemiDoc XRS+ system (BioRad).

Primary antibodies used:

1) AHR polyclonal mouse antibody A3, sc-133088-X, 1:5000 (Santa Cruz Biotechnology)
2) Anti-p53 mouse monoclonal antibody DO-1, 1:1000 (Eurogenetics)
3) Actin polyclonal goat antibody I-19, sc-1616, 1:5000 (Santa Cruz Biotechnology)

Secondary antibodies used:

1) Horseradish peroxidase-conjugated goat antimouse antibody, sc-2005, 1:5000 (Santa Cruz Biotechnology)
2) Horseradish peroxidase-conjugated mouse antirabbit antibody, sc- 2357, 1:5000 (Santa Cruz Biotechnology)

3.2 RESULTS AND DISCUSSION

3.2.1 Determination of *AHR* expression and its pathway activity

In order to uncover the possible interplay of AHR and p53, we first aimed to characterize the expression pattern of AHR in PIDA cell lines. Using RT-qPCR, we found that AHR expression varies highly among different cell lines. Namely, the lowest expression was observed in AsPc1-WT cells (Fig.6). BxPc-3 cells exhibited 14-fold higher, although statistically insignificant, AHR expression. In case of Su86.86 and Capan-1, AHR expression was 53- and 86-fold higher, respectively. Importantly, there was a significant difference in AHR expression between BxPc-3 and Su86.86. This finding is not surprising, as varying expression of AHR has been reported previously even in cells originating from the same tissue (Liu et al., 2013). We had previously acquired an AsPc1 cell line (AsPc1-KO) from our project partner, where AHR was knocked out with CRISPR-Cas9 technology. Surprisingly, this cell line exhibited a significantly higher level of AHR expression than its wild-type counterpart. Detection of AHR mRNA in this cell line can be explained by the fact that the designed primers recognized AHR mRNA upstream from the gRNA binding site. Additionally, the higher expression is somewhat expected as the knockout cell line lacks the negative feedback loop induced by AHR via AHRR.

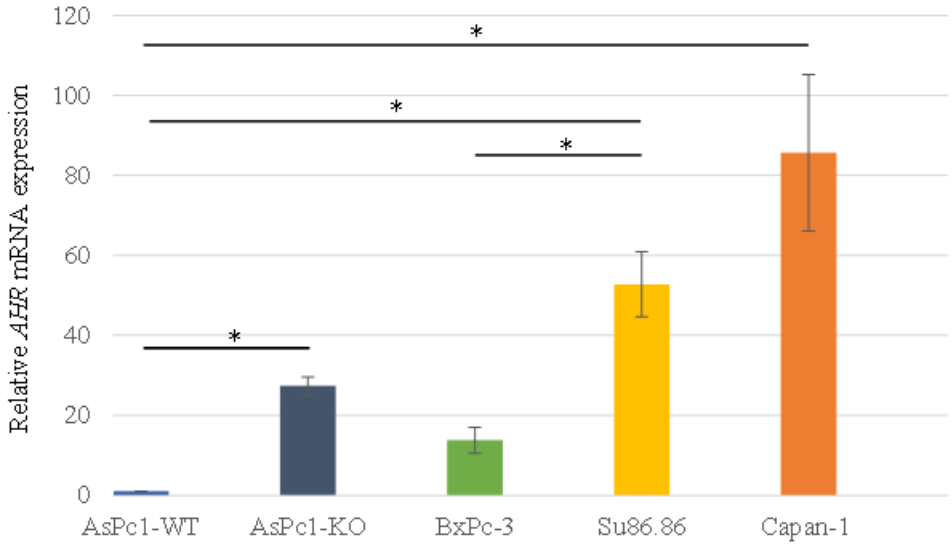

Figure 6. ***AHR* mRNA expression in different PIDA cell lines.** Gene expression was measured by RT-qPCR, normalised to the reference gene *TBP* and expressed as fold change relative to AsPc1-WT. n=3; *p-value<0,05.

AHR is a ligand-dependent transcription factor that, in addition to exogenous ligands, can be also activated by endogenous ligands synthesized by the cells themselves. Conversely,

even high expression of AHR may not necessarily result in its activity and therefore influence on gene expression. Considering this, it was our next goal to analyse the activity of AHR pathway. A good surrogate for this is *CYP1A1*, which is under tight control of AHR. RT-qPCR analysis revealed that *CYP1A1* is expressed in all cell lines that have AHR protein (Fig.7). In addition, there seemed to be a positive correlation between *AHR* (Fig.6) and *CYP1A1* expression (Fig.7) as the highest *AHR*-expressing cell lines Su86.86 and Capan-1 exhibited a trend of higher *CYP1A1* expression. This was somewhat expected, as *CYP1A1* expression is known to be dependent on AHR activity/levels. Importantly, we were unable to detect *CYP1A1* expression in AsPc1-KO cells. This further corroborates that these cells indeed lack AHR and reinforces our knowledge on CYP1A1 being tightly regulated by AHR.

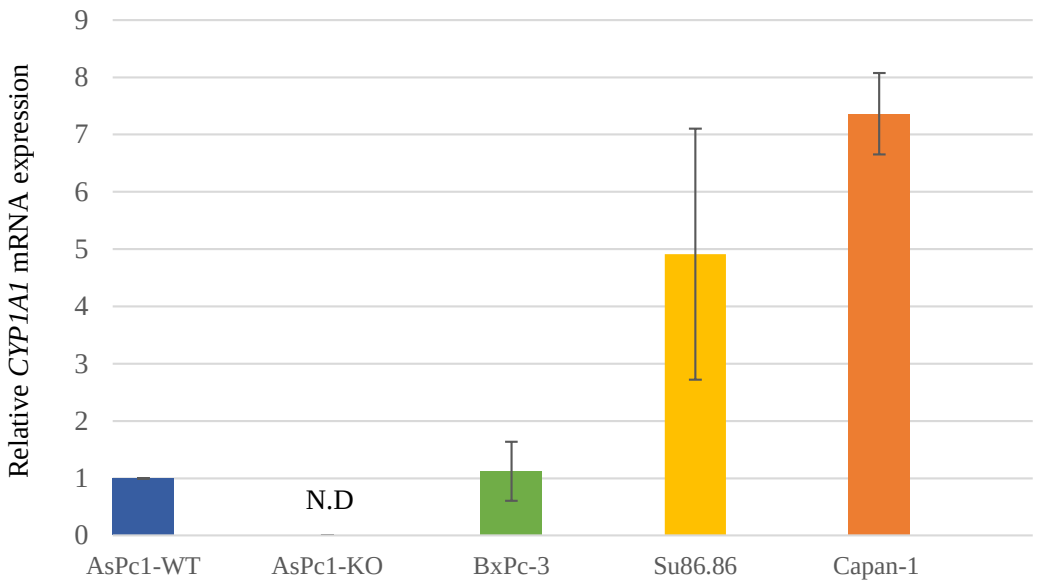

Figure 7. *CYP1A1* mRNA expression in different PIDA cell lines. Gene expression was measured by RT-qPCR, normalised to the reference gene *TBP* and expressed as fold change relative to AsPc1-WT. n=2; N.D – Not detected.

3.2.2 Analysis of *TP53* expression in PIDA cell lines

The tumour suppressor p53 is mutated and thus inactivated in many cancers. Regardless, its mRNA expression pattern can be expected to remain unchanged. Therefore, to elaborate the possible interplay between *AHR* and *TP53*, we next aimed to determine *TP53* expression in our selected PIDA cell lines. RT-qPCR analysis demonstrates that AsPc1-WT, BxPc-3 and Su86.86 express *TP53* at similar levels (Fig.8). Interestingly, the AsPc1-KO cell line lacking

AHR exhibited a 35-fold reduction in *TP53* expression when compared to its wild-type counterpart. This positive correlation is in opposition of previous publication, where negative correlation was observed in breast carcinomas (Z. D. Li et al., 2014). Yet, consistent with previous findings in breast carcinomas was the expression of *TP53* in Capan-1 cell line. More specifically, there was a negative correlation between AHR and p53 expression – i.e., high AHR - low p53 in Capan-1 and *vice versa* in AsPc-WT. However, in select cell lines p53 gene is mutated, which causes altered functionality. Nevertheless, these findings can show signs of direct or indirect modulation of p53 expression by AHR signalling pathway, but no conclusions can yet be made. Subsequent analyses should be carried out to be able to explain this phenomenon better.

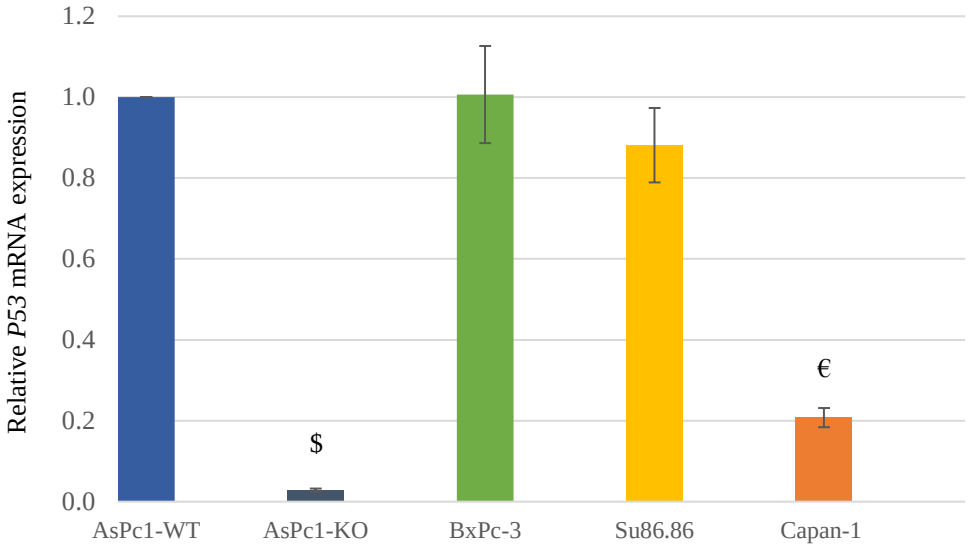

Figure 8. *TP53* mRNA expression in different PIDA cell lines. Gene expression was measured by RT-qPCR, normalised to the reference gene *TBP* and expressed as fold change relative to AsPc1-WT. n=3; $,€ p<0,05 vs other cell lines.

3.2.3 Determination of AHR and p53 protein levels in PIDA cell lines

It is known that mRNA expression does not necessarily have to correlate with protein levels. For example, the amounts of mRNA and protein can be influenced by their stability and regulation of translation. Considering that proteins are the actual "workhorses" of the cell, it was our next goal to assess AHR and p53 protein levels in PIDA cell lines. Western blot analysis indicated that both BxPc-3 (10,5-fold) and Su86.86 (11,9-fold) have higher amounts of AHR than AsPc1-WT (Fig.9). Additionally, we did not detect AHR protein in AsPc1-KO

cells, corroborating that they indeed are AHR-deficient. Importantly, in Capan-1 cells, AHR protein amount was comparable to AsPc1-WT. This contradicts our mRNA analysis, where we observed an 86-fold higher *AHR* expression in Capan-1 compared to AsPc1-WT (Fig.6). Although the exact mechanism for this remains elusive, it is reasonable to hypothesise that the translation efficiency of AHR protein could be downregulated or degradation increased in Capan-1 cells.

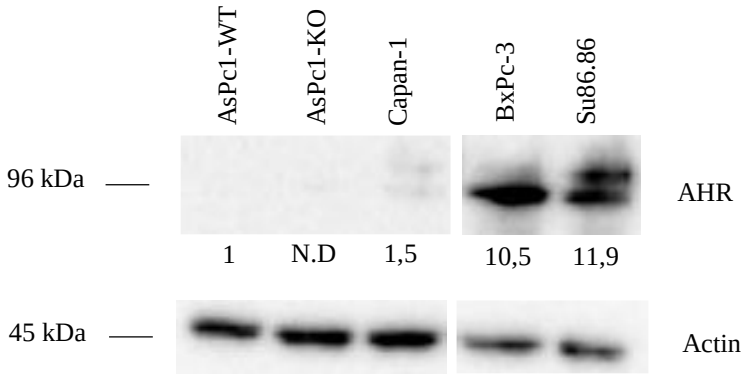

Figure 9. AHR protein levels in PIDA cell lines. Western blot analysis was performed on a PVDF membrane with the AHR antibody and ß-actin as a loading control. Densitometry analysis result is shown as protein amounts normalized with actin and presented relative to AsPc1-WT. n=1

Analysis of p53 protein levels in different PIDA cell lines revealed that AHR-deficient cells almost lack p53 (Fig.10). This in accordance with RT-qPCR experiments where we detected significantly lower amounts of *TP53* mRNA in AsPc1-KO than in its wild-type counterpart. BxPc-3 and Su86.86 seemed to have higher amounts of p53 when compared to AsPc1-WT, although their mRNA expression was not different. However, additional replicate experiments are necessary to draw any final conclusions. The amount of p53 in Capan-1 seemed to correlate with its mRNA expression as in both cases - it was lower than in AsPc1-WT, BxPc-3 and Su86.86 cells.

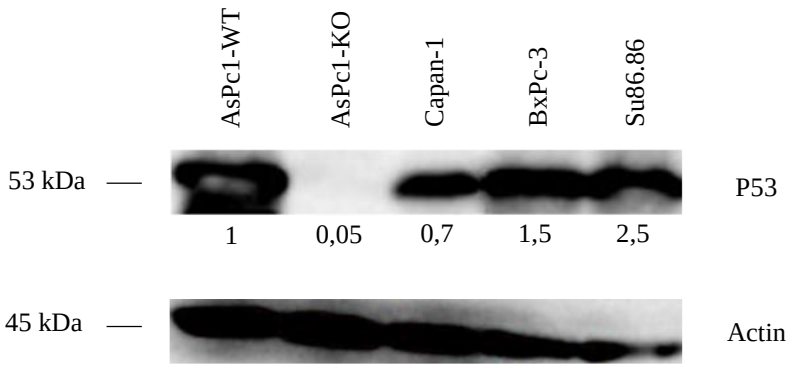

Figure 10. p53 protein levels in PIDA cell lines. Western blot analysis was performed on a PVDF membrane with the p53 antibody and ß-actin as a loading control. Densitometry analysis result is shown as protein amounts normalized with actin and presented relative to AsPc1-WT. n=1

Taken together, we have characterized the mRNA expression and protein levels of AHR and p53 in five different cell lines. We observed some correlation between AHR and p53, although additional experiments are needed to corroborate these findings. These results are a good foundation for future experiments. The use of AHR activators and inhibitors is planned to clarify the possible interplay between AHR and p53 in pancreatic invasive ductal adenocarcinoma cells in subsequent experiments.

SUMMARY

Present study can be summarized as follows:

1. *AHR* mRNA levels are variable within cell-lines derived from the same tissue and is supported by previous research. Gene knock-out PIDA cell line have higher AHR expression. It can possibly be explained due to inability of the protein to self-regulate by AHRR.

   Analysis of the AHR pathway downstream target CYP1A1 showed a positive correlation with AHR mRNA levels. Notably, AHR KO cell line had non-detectable CYP1A1 mRNA levels. This, in turn, strengthens importance of AHR in regulation of CYP1A1.
2. Expression patterns of *TP53* in PIDA cell lines were shown to have negative correlation with *AHR* levels in breast cancer– i.e., higher amounts of *AHR* mRNA correspond to lower p53 mRNA levels. Our results partially support this correlation. AHR knock-out cell line does not express detectable level of p53 mRNA.
3. To ensure the discoveries from two previous aims, protein level analysis was performed. As expected from previous results, AHR-KO cell line p53 protein levels were undetectable. Capan-1 could be hypothesized to have a reduced rate of AHR translation, due to high difference in relative mRNA and protein levels. However, replicate experiments are required to draw substantial conclusions.

REFERENCES

Adachi, J., Mori, Y., Matsui, S., Takigami, H., Fujino, J., Kitagawa, H., Miller, C. A., Kato, T., Saeki, K., & Matsuda, T. (2001). Indirubin and indigo are potent aryl hydrocarbon receptor ligands present in human urine. *The Journal of Biological Chemistry*, *276*(34), 31475–31478. https://doi.org/10.1074/JBC.C100238200

Ambolet-Camoit, A., Bui, L. C., Pierre, S., Chevallier, A., Marchand, A., Coumoul, X., Garlatti, M., Andreau, K., Barouki, R., & Aggerbeck, M. (2010). 2,3,7,8-tetrachlorodibenzo-p-dioxin counteracts the p53 response to a genotoxicant by upregulating expression of the metastasis marker agr2 in the hepatocarcinoma cell line HepG2. *Toxicological Sciences : An Official Journal of the Society of Toxicology*, *115*(2), 501–512. https://doi.org/10.1093/TOXSCI/KFQ082

American Cancer Society. (2022). *Cancer Facts & Figures 2022*. American Cancer Society. https://www.cancer.org/cancer/pancreatic-cancer/detection-diagnosis-staging/survival-rates.html#references

Baba, T., Mimura, J., Gradin, K., Kuroiwa, A., Watanabe, T., Matsuda, Y., Inazawa, J., Sogawa, K., & Fujii-Kuriyama, Y. (2001). Structure and expression of the Ah receptor repressor gene. *The Journal of Biological Chemistry*, *276*(35), 33101–33110. https://doi.org/10.1074/JBC.M011497200

Backlund, M., & Ingelman-Sundberg, M. (2004). Different structural requirements of the ligand binding domain of the aryl hydrocarbon receptor for high- and low-affinity ligand binding and receptor activation. *Molecular Pharmacology*, *65*(2), 416–425. https://doi.org/10.1124/MOL.65.2.416

Badawi, A. F., Cavalieri, E. L., & Rogan, E. G. (2001). Role of human cytochrome P450 1A1, 1A2, 1B1, and 3A4 in the 2-, 4-, and 16alpha-hydroxylation of 17beta-estradiol. *Metabolism: Clinical and Experimental*, *50*(9), 1001–1003. https://doi.org/10.1053/META.2001.25592

Baker, S. J., Fearon, E. R., Nigro, J. M., Hamilton, S. R., Preisinger, A. C., Jessup, J. M., Vantuinen, P., Ledbetter, D. H., Barker, D. F., Nakamura, Y., White, R., & Vogelstein, B. (1989). Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science (New York, N.Y.)*, *244*(4901), 217–221. https://doi.org/10.1126/SCIENCE.2649981

Bargonetti, J., Manfredi, J. J., Chen, X., Marshak, D. R., & Prives, C. (1993). A proteolytic fragment from the central region of p53 has marked sequence-specific DNA-binding activity when generated from wild-type but not from oncogenic mutant p53 protein. *Genes & Development*, *7*(12b), 2565–2574. https://doi.org/10.1101/GAD.7.12B.2565

Bell, D., Berchuck, A., Birrer, M., Chien, J., Cramer, D. W., Dao, F., Dhir, R., Disaia, P., Gabra, H., Glenn, P., Godwin, A. K., Gross, J., Hartmann, L., Huang, M., Huntsman, D. G., Iacocca, M., Imielinski, M., Kalloger, S., Karlan, B. Y., … Thomson, E. (2011). Integrated genomic analyses of ovarian carcinoma. *Nature 2011 474:7353*, *474*(7353), 609–615. https://doi.org/10.1038/nature10166

Bell, D. R., & Poland, A. (2000). Binding of aryl hydrocarbon receptor (AhR) to AhR-interacting protein. The role of hsp90. *The Journal of Biological Chemistry*, *275*(46), 36407–36414. https://doi.org/10.1074/JBC.M004236200

Belyi, V. A., Ak, P., Markert, E., Wang, H., Hu, W., Puzio-Kuter, A., & Levine, A. J. (2010). The Origins and Evolution of the p53 Family of Genes. *Cold Spring Harbor Perspectives in Biology*, *2*(6), a001198–a001198. https://doi.org/10.1101/cshperspect.a001198

Benchimol, S., Lamb, P., Crawford, L. v., Sheer, D., Shows, T. B., Bruns, G. A. P., & Peacock, J. (1985). Transformation associated p53 protein is encoded by a gene on human chromosome 17. *Somatic Cell and Molecular Genetics*, *11*(5), 505–510. https://doi.org/10.1007/BF01534845

Bienz, B., Zakut-Houri, R., Givol, D., & Oren, M. (1984). Analysis of the gene coding for the murine cellular tumour antigen p53. *The EMBO Journal*, *3*(9), 2179–2183. https://doi.org/10.1002/J.1460-2075.1984.TB02110.X

Black, V. H., & Quattrochi, L. C. (2004). Molecular cloning of the guinea pig CYP1A2 gene 5'-flanking region: identification of functional aromatic hydrocarbon response element and characterization of CYP1A2 expression in GPC16 cells. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, *32*(6), 595–602. https://doi.org/10.1124/DMD.32.6.595

Bourdon, J.-C., Fernandes, K., Murray-Zmijewski, F., Liu, G., Diot, A., Xirodimas, D. P., Saville, M. K., & Lane, D. P. (2005). p53 isoforms can regulate p53 transcriptional activity. *Genes & Development*, *19*(18), 2122–2137. https://doi.org/10.1101/gad.1339905

Dai, C., & Gu, W. (2010). p53 post-translational modification: deregulated in tumorigenesis. *Trends in Molecular Medicine*, *16*(11), 528–536. https://doi.org/10.1016/J.MOLMED.2010.09.002

Deleo, A. B., Jayt, G., Appellat, E., Duboist, G. C., Lawt, L. W., & Old, L. J. (1979). *Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse (tumor antigen/immunoprecipitation/p53)* (Vol. 76, Issue 5).

Denison, M. S., Fisher, J. M., & Whitlock, J. P. (1988). The DNA recognition site for the dioxin-Ah receptor complex. Nucleotide sequence and functional analysis. *Journal of Biological Chemistry*, *263*(33), 17221–17224. https://doi.org/10.1016/S0021-9258(19)77819-3

Eischen, C. M. (2016). Genome Stability Requires p53. *Cold Spring Harbor Perspectives in Medicine*, *6*(6), a026096. https://doi.org/10.1101/CSHPERSPECT.A026096

El-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W., & Vogelstein, B. (1992). Definition of a consensus binding site for p53. *Nature Genetics 1992 1:1*, *1*(1), 45–49. https://doi.org/10.1038/ng0492-45

Eliyahu, D., Michalovitz, D., & Oren, M. (1985). Overproduction of p53 antigen makes established cells highly tumorigenic. *Nature*, *316*(6024), 158–160. https://doi.org/10.1038/316158A0

Eliyahu, D., Raz, A., Gruss, P., Givol, D., & Oren, M. (1984). Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. *Nature*, *312*(5995), 646–649. https://doi.org/10.1038/312646A0

Eltom, S. E., Zhang, L., & Jefcoate, C. R. (1999). Regulation of Cytochrome P-450 (CYP) 1B1 in Mouse Hepa-1 Variant Cell Lines: A Possible Role for Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT) as a Suppressor of CYP1B1 Gene Expression. *Molecular Pharmacology*, *55*(3).

Engeland, K. (2017). Cell cycle arrest through indirect transcriptional repression by p53: I have a DREAM. *Cell Death & Differentiation 2018 25:1*, *25*(1), 114–132. https://doi.org/10.1038/cdd.2017.172

Farrell, K., Safe, L., & Safe, S. (1987). Synthesis and aryl hydrocarbon receptor binding properties of radiolabeled polychlorinated dibenzofuran congeners. *Archives of Biochemistry and Biophysics*, *259*(1), 185–195. https://doi.org/10.1016/0003-9861(87)90485-1

Fischer, N. W., Prodeus, A., Malkin, D., & Gariépy, J. (2016). p53 oligomerization status modulates cell fate decisions between growth, arrest and apoptosis. *Cell Cycle*, *15*(23), 3210–3219. https://doi.org/10.1080/15384101.2016.1241917/SUPPL_FILE/KCCY_A_1241917_SM0990.ZIP

Frauenstein, K., Sydlik, U., Tigges, J., Majora, M., Wiek, C., Hanenberg, H., Abel, J., Esser, C., Fritsche, E., Krutmann, J., & Haarmann-Stemmann, T. (2013). Evidence for a novel anti-apoptotic pathway in human keratinocytes involving the aryl hydrocarbon receptor, E2F1, and checkpoint kinase 1. *Cell Death and Differentiation*, *20*(10), 1425–1434. https://doi.org/10.1038/CDD.2013.102

Fritsche, E., Schäfer, C., Calles, C., Bernsmann, T., Bernshausen, T., Wurm, M., Hübenthal, U., Cline, J. E., Hajimiragha, H., Schroeder, P., Klotz, L. O., Rannug, A., Fürst, P., Hanenberg, H., Abel, J., & Krutmann, J. (2007). Lightening up the UV response by identification of the arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(21), 8851–8856. https://doi.org/10.1073/PNAS.0701764104

Großkopf, H., Walter, K., Karkossa, I., von Bergen, M., & Schubert, K. (2021). Non-Genomic AhR-Signaling Modulates the Immune Response in Endotoxin-Activated Macrophages After Activation by the Environmental Stressor BaP. *Frontiers in Immunology*, *12*, 1008. https://doi.org/10.3389/FIMMU.2021.620270/BIBTEX

Gualtieri, M., Øvrevik, J., Mollerup, S., Asare, N., Longhin, E., Dahlman, H. J., Camatini, M., & Holme, J. A. (2011). Airborne urban particles (Milan winter-PM2.5) cause mitotic arrest and cell death: Effects on DNA, mitochondria, AhR binding and spindle organization. *Mutation Research*, *713*(1–2), 18–31. https://doi.org/10.1016/J.MRFMMM.2011.05.011

Hafner, A., Bulyk, M. L., Jambhekar, A., & Lahav, G. (2019). The multiple mechanisms that regulate p53 activity and cell fate. *Nature Reviews Molecular Cell Biology*, *20*. https://doi.org/10.1038/s41580-019-0110-x

Heid, S. E., Pollenz, R. S., & Swanson, H. I. (2000). Role of Heat Shock Protein 90 Dissociation in Mediating Agonist-Induced Activation of the Aryl Hydrocarbon Receptor. *Molecular Pharmacology*, *57*(1).

Hockings, J. K., Thorne, P. A., Kemp, M. Q., Morgan, S. S., Selmin, O., & Romagnolo, D. F. (2006). The ligand status of the aromatic hydrocarbon receptor modulates transcriptional activation of BRCA-1 promoter by estrogen. *Cancer Research*, *66*(4), 2224–2232. https://doi.org/10.1158/0008-5472.CAN-05-1619

Hu, W., Zhao, J., & Pei, G. (2013). Activation of aryl hydrocarbon receptor (ahr) by tranilast, an anti-allergy drug, promotes miR-302 expression and cell reprogramming. *The Journal of Biological Chemistry*, *288*(32), 22972–22984. https://doi.org/10.1074/JBC.M113.475624

Ikuta, T., Eguchi, H., Tachibana, T., Yoneda, Y., & Kawajiri, K. (1998). Nuclear Localization and Export Signals of the Human Aryl Hydrocarbon Receptor *. *Journal of Biological Chemistry*, *273*(5), 2895–2904. https://doi.org/10.1074/JBC.273.5.2895

Ishida, M., Mikami, S., Shinojima, T., Kosaka, T., Mizuno, R., Kikuchi, E., Miyajima, A., Okada, Y., & Oya, M. (2015). Activation of aryl hydrocarbon receptor promotes invasion of clear cell renal cell carcinoma and is associated with poor prognosis and cigarette smoke. *International Journal of Cancer*, *137*(2), 299–310. https://doi.org/10.1002/IJC.29398

Jain, A. K., & Barton, M. C. (2018). P53: Emerging roles in stem cells, development and beyond. *Development (Cambridge)*, *145*(8). https://doi.org/10.1242/DEV.158360/19342

Jeffrey, P. D., Gorina, S., & Pavletich, N. P. (1995). Crystal Structure of the Tetramerization Domain of the p53 Tumor Suppressor at 1.7 Angstroms. *Science*, *267*(5203), 1498–1502. https://doi.org/10.1126/SCIENCE.7878469

Jenkins, J. R., Rudge, K., & Currie, G. A. (1984). Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. *Nature*, *312*(5995), 651–654. https://doi.org/10.1038/312651A0

Jin, U. H., Kim, S. B., & Safe, S. (2015). Omeprazole Inhibits Pancreatic Cancer Cell Invasion Through a Non-genomic Aryl Hydrocarbon Receptor Pathway. *Chemical Research in Toxicology*, *28*(5), 907. https://doi.org/10.1021/TX5005198

Jones, G. G., Reaper, P. M., Pettitt, A. R., & Sherrington, P. D. (2004). The ATR-p53 pathway is suppressed in noncycling normal and malignant lymphocytes. *Oncogene 2004 23:10*, *23*(10), 1911–1921. https://doi.org/10.1038/sj.onc.1207318

Jones, J. M., & Anderson, J. W. (1999). Relative potencies of PAHs and PCBs based on the response of human cells. *Environmental Toxicology and Pharmacology*, *7*(1), 19–26. https://doi.org/10.1016/S1382-6689(98)00045-3

Kandoth, C., McLellan, M. D., Vandin, F., Ye, K., Niu, B., Lu, C., Xie, M., Zhang, Q., McMichael, J. F., Wyczalkowski, M. A., Leiserson, M. D. M., Miller, C. A., Welch, J. S., Walter, M. J., Wendl, M. C., Ley, T. J., Wilson, R. K., Raphael, B. J., & Ding, L. (2013a). Mutational landscape and significance across 12 major cancer types. *Nature 2013 502:7471*, *502*(7471), 333–339. https://doi.org/10.1038/nature12634

Katiyar, S. K., Matsui, M. S., & Mukhtar, H. (2000). Ultraviolet-B exposure of human skin induces cytochromes P450 1A1 and 1B1. *The Journal of Investigative Dermatology*, *114*(2), 328–333. https://doi.org/10.1046/J.1523-1747.2000.00876.X

Kaye, J., Piryatinsky, V., Birnberg, T., Hingaly, T., Raymond, E., Kashi, R., Amit-Romach, E., Caballero, I. S., Towfic, F., Ator, M. A., Rubinstein, E., Laifenfeld, D., Orbach, A., Shinar, D., Marantz, Y., Grossman, I., Knappertz, V., Hayden, M. R., & Laufer, R. (2016). Laquinimod arrests experimental autoimmune encephalomyelitis by activating the aryl hydrocarbon receptor. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(41), E6145–E6152. https://doi.org/10.1073/PNAS.1607843113

Kazlauskas, A., Poellinger, L., & Pongratz, I. (1999). Evidence That the Co-chaperone p23 Regulates Ligand Responsiveness of the Dioxin (Aryl Hydrocarbon) Receptor. *Journal of Biological Chemistry*, *274*(19), 13519–13524. https://doi.org/10.1074/JBC.274.19.13519

Knudson, A. G. (1971). Mutation and cancer: statistical study of retinoblastoma. *Proceedings of the National Academy of Sciences of the United States of America*, *68*(4), 820–823. https://doi.org/10.1073/PNAS.68.4.820

Ko, C. I., Fan, Y., de Gannes, M., Wang, Q., Xia, Y., & Puga, A. (2016). Repression of the Aryl Hydrocarbon Receptor Is Required to Maintain Mitotic Progression and Prevent Loss of Pluripotency of Embryonic Stem Cells. *Stem Cells (Dayton, Ohio)*, *34*(12), 2825–2839. https://doi.org/10.1002/STEM.2456

Koboldt, D. C., Fulton, R. S., McLellan, M. D., Schmidt, H., Kalicki-Veizer, J., McMichael, J. F., Fulton, L. L., Dooling, D. J., Ding, L., Mardis, E. R., Wilson, R. K., Ally, A., Balasundaram, M., Butterfield, Y. S. N., Carlsen, R., Carter, C., Chu, A., Chuah, E., Chun, H. J. E., … Palchik, J. D. (2012). Comprehensive molecular portraits of human breast tumours. *Nature 2012 490:7418*, *490*(7418), 61–70. https://doi.org/10.1038/nature11412

Koliopanos, A., Kleeff, J., Xiao, Y., Safe, S., Zimmermann, A., Büchler, M. W., & Friess, H. (2002). Increased arylhydrocarbon receptor expression offers a potential therapeutic target for pancreatic cancer. *Oncogene*, *21*(39), 6059–6070. https://doi.org/10.1038/SJ.ONC.1205633

Kress, M., May, E., Cassingena, R., & May, P. (1979). Simian virus 40-transformed cells express new species of proteins precipitable by anti-simian virus 40 tumor serum. *Journal of Virology*, *31*(2), 472. https://doi.org/10.1128/JVI.31.2.472-483.1979

Kumar, M. B., & Perdew, G. H. (1999). Nuclear Receptor Coactivator SRC-1 Interacts With the Q-Rich Subdomain of the AhR and Modulates its Transactivation Potential. *Gene Expression*, *8*(5–6), 273. /pmc/articles/PMC6157383/

Kumar, M. B., Tarpey, R. W., & Perdew, G. H. (1999). Differential Recruitment of Coactivator RIP140 byAh and Estrogen Receptors: ABSENCE OF A ROLE FOR LXXLL MOTIFS. *Journal of Biological Chemistry*, *274*(32), 22155–22164. https://doi.org/10.1074/JBC.274.32.22155

Lamb, P., & Crawford, L. (1986). Characterization of the human p53 gene. *Molecular and Cellular Biology*, *6*(5), 1379–1385. https://doi.org/10.1128/MCB.6.5.1379-1385.1986

Lane, D. P., & Crawford, L. v. (1979). T antigen is bound to a host protein in SY40-transformed cells. *Nature 1979 278:5701*, *278*(5701), 261–263. https://doi.org/10.1038/278261a0

Latchney, S. E., Lioy, D. T., Henry, E. C., Gasiewicz, T. A., Strathmann, F. G., Mayer-Pröschel, M., & Opanashuk, L. A. (2011). Neural precursor cell proliferation is disrupted through activation of the aryl hydrocarbon receptor by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Stem Cells and Development*, *20*(2), 313–326. https://doi.org/10.1089/SCD.2009.0529

Lee, J. eun, Cho, S. G., Ko, S. G., Ahrmad, S. A., Puga, A., & Kim, K. (2020). Regulation of a long noncoding RNA MALAT1 by aryl hydrocarbon receptor in pancreatic cancer

cells and tissues. *Biochemical and Biophysical Research Communications*, *532*(4), 563–569. https://doi.org/10.1016/J.BBRC.2020.08.053

Lees, M. J., & Whitelaw, M. L. (1999). Multiple roles of ligand in transforming the dioxin receptor to an active basic helix-loop-helix/PAS transcription factor complex with the nuclear protein Arnt. *Molecular and Cellular Biology*, *19*(8), 5811–5822. https://doi.org/10.1128/MCB.19.8.5811

Levine, A. J. (1997). p53, the Cellular Gatekeeper for Growth and Division. *Cell*, *88*(3), 323–331. https://doi.org/10.1016/S0092-8674(00)81871-1

Li, F. P., & Fraumeni, J. F. (1969). Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? *Annals of Internal Medicine*, *71*(4), 747–752. https://doi.org/10.7326/0003-4819-71-4-747

Li, L., Chen, H., Gao, Y., Wang, Y. W., Zhang, G. Q., Pan, S. H., Ji, L., Kong, R., Wang, G., Jia, Y. H., Bai, X. W., & Sun, B. (2016). Long Noncoding RNA MALAT1 Promotes Aggressive Pancreatic Cancer Proliferation and Metastasis via the Stimulation of Autophagy. *Molecular Cancer Therapeutics*, *15*(9), 2232–2243. https://doi.org/10.1158/1535-7163.MCT-16-0008

Li, Z. D., Wang, K., Yang, X. W., Zhuang, Z. G., Wang, J. J., & Tong, X. W. (2014). Expression of aryl hydrocarbon receptor in relation to p53 status and clinicopathological parameters in breast cancer. *International Journal of Clinical and Experimental Pathology*, *7*(11), 7931–7937. https://pubmed.ncbi.nlm.nih.gov/25550834/

Lin, J., Chen, J., Elenbaas, B., & Levine, A. J. (1994). *Several hydrophobic amino acids in the p53 amino-terminal domain are re.qmred for transcriptional activation, binding to mdm-2 and the adenovirus 5 E1B 55-kD protein*.

Linzer, D. I. H., & Levine, A. J. (1979). Characterization of a 54K Dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell*, *17*(1), 43–52. https://doi.org/10.1016/0092-8674(79)90293-9

Liu, Z., Wu, X., Zhang, F., Han, L., Bao, G., He, X., & Xu, Z. (2013). AhR expression is increased in hepatocellular carcinoma. *Journal of Molecular Histology*, *44*(4), 455–461. https://doi.org/10.1007/S10735-013-9495-6

Longnecker, D. S. (2021). Anatomy and Histology of the Pancreas. *Pancreapedia: The Exocrine Pancreas Knowledge Base*. https://doi.org/10.3998/PANC.2021.01

Ma, Q., & Baldwin, K. T. (2000). 2,3,7,8-Tetrachlorodibenzo-p-dioxin-induced Degradation of Aryl Hydrocarbon Receptor (AhR) by the Ubiquitin-Proteasome Pathway: ROLE OF THE TRANSCRIPTION ACTIVATON AND DNA BINDING OF AhR. *Journal of Biological Chemistry*, *275*(12), 8432–8438. https://doi.org/10.1074/JBC.275.12.8432

Ma, Q., Dong, L., & Whitlock, J. P. (1995). Transcriptional Activation by the Mouse Ah Receptor.: INTERPLAY BETWEEN MULTIPLE STIMULATORY AND INHIBITORY FUNCTIONS. *Journal of Biological Chemistry*, *270*(21), 12697–12703. https://doi.org/10.1074/JBC.270.21.12697

Mallavarapu, . A, Mitchison, T., & Vignjevic, J. ; D. (1999). Supporting Online Material Aryl Hydrocarbon Receptor Antagonists Promote the Expansion of Human Hematopoietic Stem Cells. *Proc. Natl. Acad. Sci. U.S.A*, *146*, 79. https://doi.org/10.1126/science.1191710

Marshall, C. B., Beeler, J. S., Lehmann, B. D., Gonzalez-Ericsson, P., Sanchez, V., Sanders, M. E., Boyd, K. L., & Pietenpol, J. A. (2021). Tissue-specific expression of p73 and p63 isoforms in human tissues. *Cell Death & Disease 2021 12:8*, *12*(8), 1–10. https://doi.org/10.1038/s41419-021-04017-8

Matikainen, T., Perez, G. I., Jurisicova, A., Pru, J. K., Schlezinger, J. J., Ryu, H. Y., Laine, J., Sakai, T., Korsmeyer, S. J., Casper, R. F., Sherr, D. H., & Tilly, J. L. (2001). Aromatic hydrocarbon receptor-driven Bax gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. *Nature Genetics*, *28*(4), 355–360. https://doi.org/10.1038/NG575

Matsumoto, Y., Ide, F., Kishi, R., Akutagawa, T., Sakai, S., Nakamura, M., Ishikawa, T., Fujii-Kuriyama, Y., & Nakatsuru, Y. (2007). Aryl hydrocarbon receptor plays a significant role in mediating airborne particulate-induced carcinogenesis in mice. *Environmental Science & Technology*, *41*(10), 3775–3780. https://doi.org/10.1021/ES062793G

McBride, K. A., Ballinger, M. L., Killick, E., Kirk, J., Tattersall, M. H. N., Eeles, R. A., Thomas, D. M., & Mitchell, G. (2014). Li-Fraumeni syndrome: cancer risk assessment and clinical management. *Nature Reviews Clinical Oncology 2014 11:5*, *11*(5), 260–271. https://doi.org/10.1038/nrclinonc.2014.41

McGuire, J., Whitelaw, M. L., Pongratz, I., Gustafsson, J. A., & Poellinger, L. (1994). A cellular factor stimulates ligand-dependent release of hsp90 from the basic helix-loop-

helix dioxin receptor. *Molecular and Cellular Biology*, *14*(4), 2438–2446. https://doi.org/10.1128/MCB.14.4.2438-2446.1994

Melero, JoséA., Stitt, D. T., Mangel, W. F., & Carroll, R. B. (1979). Identification of new polypeptide species (48–55K) immunoprecipitable by antiserum to purified large T antigen and present in SV40-infected and -transformed cells. *Virology*, *93*(2), 466–480. https://doi.org/10.1016/0042-6822(79)90250-2

Mezrich, J. D., Fechner, J. H., Zhang, X., Johnson, B. P., Burlingham, W. J., & Bradfield, C. A. (2010). An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *Journal of Immunology (Baltimore, Md. : 1950)*, *185*(6), 3190–3198. https://doi.org/10.4049/JIMMUNOL.0903670

Mijit, M., Caracciolo, V., Melillo, A., Amicarelli, F., & Giordano, A. (2020). Role of p53 in the Regulation of Cellular Senescence. *Biomolecules*, *10*(3). https://doi.org/10.3390/BIOM10030420

Nigro, J. M., Baker, S. J., Preisinger, A. C., Jessup, J. M., Hosteller, R., Cleary, K., Bigner, S. H., Davidson, N., Baylin, S., Devilee, P., Glover, T., Collins, F. S., Weslon, A., Modali, R., Harris, C. C., & Vogelstein, B. (1989). Mutations in the p53 gene occur in diverse human tumour types. *Nature*, *342*(6250), 705–708. https://doi.org/10.1038/342705A0

Opitz, C. A., Litzenburger, U. M., Sahm, F., Ott, M., Tritschler, I., Trump, S., Schumacher, T., Jestaedt, L., Schrenk, D., Weller, M., Jugold, M., Guillemin, G. J., Miller, C. L., Lutz, C., Radlwimmer, B., Lehmann, I., von Deimling, A., Wick, W., & Platten, M. (2011). An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature*, *478*(7368), 197–203. https://doi.org/10.1038/NATURE10491

Ozaki, T., & Nakagawara, A. (2011). Role of p53 in Cell Death and Human Cancers. *Cancers*, *3*(1), 994. https://doi.org/10.3390/CANCERS3010994

Pang, E. J., Yang, R., Fu, X. bo, & Liu, Y. fu. (2015). Overexpression of long non-coding RNA MALAT1 is correlated with clinical progression and unfavorable prognosis in pancreatic cancer. *Tumour Biology : The Journal of the International Society for Oncodevelopmental Biology and Medicine*, *36*(4), 2403–2407. https://doi.org/10.1007/S13277-014-2850-8

Parada, L. F., Land, H., Weinberg, R. A., Wolf, D., & Rotter, V. (1984). Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation. *Nature*, *312*(5995), 649–651. https://doi.org/10.1038/312649A0

Pavletich, N. P., Chambers, K. A., & Pabo, C. O. (1993). The DNA-binding domain of p53 contains the four conserved regions and the major mutation hot spots. *Genes & Development*, *7*(12b), 2556–2564. https://doi.org/10.1101/GAD.7.12B.2556

Peifer, M., Fernández-Cuesta, L., Sos, M. L., George, J., Seidel, D., Kasper, L. H., Plenker, D., Leenders, F., Sun, R., Zander, T., Menon, R., Koker, M., Dahmen, I., Müller, C., di Cerbo, V., Schildhaus, H. U., Altmüller, J., Baessmann, I., Becker, C., … Thomas, R. K. (2012). Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. *Nature Genetics 2012 44:10*, *44*(10), 1104–1110. https://doi.org/10.1038/ng.2396

Perdew, G. H. (1988). Association of the Ah receptor with the 90-kDa heat shock protein. *Journal of Biological Chemistry*, *263*(27), 13802–13805. https://doi.org/10.1016/S0021-9258(18)68314-0

Petrulis, J. R., Kusnadi, A., Ramadoss, P., Hollingshead, B., & Perdew, G. H. (2003). The hsp90 Co-chaperone XAP2 alters importin beta recognition of the bipartite nuclear localization signal of the Ah receptor and represses transcriptional activity. *The Journal of Biological Chemistry*, *278*(4), 2677–2685. https://doi.org/10.1074/JBC.M209331200

Piskorska-Pliszczynska, J., Keys, B., Safe, S., & Newman, M. S. (1986). The cytosolic receptor binding affinities and ahh induction potencies of 29 polynuclear aromatic hydrocarbons. *Toxicology Letters*, *34*(1), 67–74. https://doi.org/10.1016/0378-4274(86)90146-3

Poland, A., Glover, E., & Kende, A. S. (1976). Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. Evidence that the binding species is receptor for induction of aryl hydrocarbon hydroxylase. *The Journal of Biological Chemistry*, *251*(16), 4936–4946.

Pollet, M., Shaik, S., Mescher, M., Frauenstein, K., Tigges, J., Braun, S. A., Sondenheimer, K., Kaveh, M., Bruhs, A., Meller, S., Homey, B., Schwarz, A., Esser, C., Douki, T., Vogel, C. F. A., Krutmann, J., & Haarmann-Stemmann, T. (2018). The AHR represses

nucleotide excision repair and apoptosis and contributes to UV-induced skin carcinogenesis. *Cell Death and Differentiation*, *25*(10), 1823–1836. https://doi.org/10.1038/S41418-018-0160-1

Powell, J. B., Goode, G. D., & Eltom, S. E. (2013). The Aryl Hydrocarbon Receptor: A Target for Breast Cancer Therapy. *Journal of Cancer Therapy*, *4*(7), 1177. https://doi.org/10.4236/JCT.2013.47137

Rahib, L., Smith, B. D., Aizenberg, R., Rosenzweig, A. B., Fleshman, J. M., & Matrisian, L. M. (2014). Projecting cancer incidence and deaths to 2030: The unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Research*, *74*(11), 2913–2921. https://doi.org/10.1158/0008-5472.CAN-14-0155/659355/P/PROJECTING-CANCER-INCIDENCE-AND-DEATHS-TO-2030-THE

Richmond, O., Ghotbaddini, M., Allen, C., Walker, A., Zahir, S., & Powell, J. B. (2014). The aryl hydrocarbon receptor is constitutively active in advanced prostate cancer cells. *PloS One*, *9*(4). https://doi.org/10.1371/JOURNAL.PONE.0095058

Rivlin, N., Brosh, R., Oren, M., & Rotter, V. (2011). Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis. *Genes & Cancer*, *2*(4), 466–474. https://doi.org/10.1177/1947601911408889

Ruijs, M. W. G., Verhoef, S., Rookus, M. A., Pruntel, R., van der Hout, A. H., Hogervorst, F. B. L., Kluijt, I., Sijmons, R. H., Aalfs, C. M., Wagner, A., Ausems, M. G. E. M., Hoogerbrugge, N., van Asperen, C. J., Gomez Garcia, E. B., Meijers-Heijboer, H., ten Kate, L. P., Menko, F. H., & van 'T Veer, L. J. (2010). TP53 germline mutation testing in 180 families suspected of Li–Fraumeni syndrome: mutation detection rate and relative frequency of cancers in different familial phenotypes. *Journal of Medical Genetics*, *47*(6), 421–428. https://doi.org/10.1136/JMG.2009.073429

Rushing, S. R., & Denison, M. S. (2002). The silencing mediator of retinoic acid and thyroid hormone receptors can interact with the aryl hydrocarbon (Ah) receptor but fails to repress Ah receptor-dependent gene expression. *Archives of Biochemistry and Biophysics*, *403*(2), 189–201. https://doi.org/10.1016/S0003-9861(02)00233-3

Safe, S., Lee, S. O., & Jin, U. H. (2013). Role of the aryl hydrocarbon receptor in carcinogenesis and potential as a drug target. *Toxicological Sciences : An Official Journal of the Society of Toxicology*, *135*(1), 1–16. https://doi.org/10.1093/TOXSCI/KFT128

Saito, R., Miki, Y., Hata, S., Takagi, K., Iida, S., Oba, Y., Ono, K., Ishida, T., Suzuki, T., Ohuchi, N., & Sasano, H. (2014). Aryl hydrocarbon receptor in breast cancer—a newly defined prognostic marker. *Hormones & Cancer*, *5*(1), 11–21. https://doi.org/10.1007/S12672-013-0160-Z

Sanchez, D., & Cheung, W. L. (2015). Pathology of pancreatic tumors. *Translational Cancer Research*, *4*(6). https://doi.org/10.21037/5993

Sato, S. (2008). Low-dose dioxins alter gene expression related to cholesterol biosynthesis, lipogenesis, and glucose metabolism through the aryl hydrocarbon receptor-mediated pathway in mouse liver. *Toxicol. Appl. Pharmacol.*, *229*, 10–19.

Schulte, K. W., Green, E., Wilz, A., Platten, M., & Daumke, O. (2017). Structural Basis for Aryl Hydrocarbon Receptor-Mediated Gene Activation. *Structure (London, England : 1993)*, *25*(7), 1025-1033.e3. https://doi.org/10.1016/J.STR.2017.05.008

Seidel, S. D., Winters, G. M., Rogers, W. J., Ziccardi, M. H., Li, V., Keser, B., & Denison, M. S. (2001). Activation of the Ah Receptor Signaling Pathway by Prostaglandins. In *J BIOCHEM MOLECULAR TOXICOLOGY* (Vol. 15, Issue 4). John Wiley & Sons, Inc.

Smith, A. E., Smith, R., & Paucha, E. (1979). Characterization of different tumor antigens present in cells transformed by simian virus 40. *Cell*, *18*(2), 335–346. https://doi.org/10.1016/0092-8674(79)90053-9

Song, Y., Li, L., Ou, Y., Gao, Z., Li, E., Li, X., Zhang, W., Wang, J., Xu, L., Zhou, Y., Ma, X., Liu, L., Zhao, Z., Huang, X., Fan, J., Dong, L., Chen, G., Ma, L., Yang, J., … Zhan, Q. (2014). Identification of genomic alterations in oesophageal squamous cell cancer. *Nature 2014 509:7498*, *509*(7498), 91–95. https://doi.org/10.1038/nature13176

Soshilov, A., & Denison, M. S. (2008). Role of the Per/Arnt/Sim Domains in Ligand-dependent Transformation of the Aryl Hydrocarbon Receptor. *The Journal of Biological Chemistry*, *283*(47), 32995. https://doi.org/10.1074/JBC.M802414200

Stommel, J. M., Marchenko, N. D., Jimenez, G. S., Moll, U. M., Hope, T. J., & Wahl, G. M. (1999). A leucine-rich nuclear export signal in the p53 tetramerization domain: regulation of subcellular localization and p53 activity by NES masking. *The EMBO Journal*, *18*(6), 1660–1672. https://doi.org/10.1093/EMBOJ/18.6.1660

Su, J.-M., Lin, P., & Chang, H. (2013). Prognostic value of nuclear translocation of aryl hydrocarbon receptor for non-small cell lung cancer. *Anticancer Research*, *33*(9), 3953–3961.

Teino Indrek. (2020). *Studies on aryl hydrocarbon receptor in the mouse granulosa cell model*.

Tohkin, M., Fukuhara, M., Elizondo, G., Tomita, S., & Gonzalez, F. J. (2000). Aryl Hydrocarbon Receptor Is Required for p300-Mediated Induction of DNA Synthesis by Adenovirus E1A. *Molecular Pharmacology*, *58*(4), 845–851. https://doi.org/10.1124/MOL.58.4.845

Walker, K. K., & Levine, A. J. (1996). Identification of a novel p53 functional domain that is necessary for efficient growth suppression. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(26), 15335–15340. https://doi.org/10.1073/PNAS.93.26.15335/ASSET/45932DFE-1D0B-4862-ADCA-96586C0AA681/ASSETS/GRAPHIC/PQ2663177005.JPEG

Watson, A. J., & Hankinson, O. (1992). Dioxin- and Ah receptor-dependent protein binding to xenobiotic responsive elements and G-rich DNA studied by in vivo footprinting. *Journal of Biological Chemistry*, *267*(10), 6874–6878. https://doi.org/10.1016/S0021-9258(19)50509-9

Wilson, C. A., Ramos, L., Villaseñor, M. R., Anders, K. H., Press, M. F., Clarke, K., Karlan, B., Chen, J. J., Scully, R., Livingston, D., Zuch, R. H., Kanter, M. H., Cohen, S., Calzone, F. J., & Slamon, D. J. (1999). Localization of human BRCA1 and its loss in high-grade, non-inherited breast carcinomas. *Nature Genetics*, *21*(2), 236–240. https://doi.org/10.1038/6029

Wu, D., Potluri, N., Kim, Y., & Rastinejad, F. (2013). Structure and Dimerization Properties of the Aryl Hydrocarbon Receptor PAS-A Domain. *Molecular and Cellular Biology*, *33*(21), 4346. https://doi.org/10.1128/MCB.00698-13

Yin, X. F., Chen, J., Mao, W., Wang, Y. H., & Chen, M. H. (2013). Downregulation of aryl hydrocarbon receptor expression decreases gastric cancer cell growth and invasion. *Oncology Reports*, *30*(1), 364–370. https://doi.org/10.3892/OR.2013.2410

Zhang, Y., Xiong, Y., & Yarbrough, W. G. (1998). ARF Promotes MDM2 Degradation and Stabilizes p53: ARF-INK4a Locus Deletion Impairs Both the Rb and p53 Tumor Suppression Pathways. *Cell*, *92*(6), 725–734. https://doi.org/10.1016/S0092-8674(00)81401-4

Websites used:

UniProt.org - https://beta.uniprot.org/uniprotkb/P35869/entry; Date accessed: 17.05.2022

Ensembl.org A - https://www.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000106546;r=7:16916359-17346152; Date accessed: 18.05.2022

Ensembl.org B - https://www.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000141510;r=17:7661779-7687538; Date accessed: 21.05.2022

Clevelandclinic.org- https://my.clevelandclinic.org/health/diseases/21652-adenocarcinoma-cancers; Date accessed: 21.05.2022

Cancer.gov - https://www.cancer.gov/publications/dictionaries/cancer-terms/def/pancreas; Date accessed: 10.05.2022

p53.free.fr - http://p53.free.fr/Database/p53_cancer/p53_pancreatic.html; Date accessed: 22.05.2022

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