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TOPIC HIGHLIGHT

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Nuclear receptors and pathogenesis of pancreatic cancer

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with a median overall survival time of 5 mo and the five years survival less than 5%, a rate essentially unchanged over the course of the years. A well defined progression model of accumulation of genetic alterations ranging from single point mutations to gross chromosomal abnormalities has been introduced to describe the origin of this disease. However, due to the its subtle nature and concurring events PDAC cure remains elusive. Nuclear receptors (NR) are members of a large superfamily of evolutionarily conserved ligand-regulated DNA-binding transcription factors functionally involved in important cellular functions ranging from regulation of metabolism, to growth and development. Given the nature of their ligands, NR are very tempting drug targets and their pharmacological modulation has been widely exploited for the treatment of metabolic and inflammatory diseases. There are now clear evidences that both classical ligand-activated and orphan NR are involved in the pathogenesis of PDAC from its very early stages; nonetheless many aspects of their role are not fully understood. The purpose of this review is to highlight the striking connections that link peroxisome proliferator activated receptors, retinoic acid receptors, retinoid X receptor, androgen receptor, estrogen receptors and the orphan NR Nur, chicken ovalbumin upstream promoter transcription factor Ⅱ and the liver receptor homologue-1 receptor to PDAC development, connections that could lead to the identification of novel therapies for this disease.

Key words: Peroxisome proliferator activated receptor; Pancreatic intraepithelial neoplasia; COUP-TFⅡ; Nuclear receptors; Orphan nuclear receptor; Nuclear receptors 4A2; Nuclear receptors 2F2; Pancreatic cancer; Retinoid X receptor; Testicular receptor 3

Core tip: Pancreatic cancer is a devastating disease with well defined genetic alterations made deadly by its subtle nature and the lack of effective drugs. Nuclear receptors (NR) are ligand-regulated transcription factors involved in important cellular functions and tempting targets for drug development. There are now evidences that classical ligand-activated peroxisome proliferator activated receptors, retinoic acid receptors, retinoid X receptors, androgen receptor, estrogen receptors and orphan Nur, chicken ovalbumin upstream promoter transcription factor Ⅱ and liver receptor homologue-1 NR are involved in the pathogenesis of pancreatic cancer. No clinical application of these NR in pancreatic cancer cure is reported but a more comprehensive analysis of NR action could lead to the identification of new treatments for this disease.

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INTRODUCTION

The most frequent form of pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC) one of the most lethal cancer and the fifth cause of cancer death in the Developed Countries[1]. Treatment of PDAC is primarily a combination of curative surgery and adjuvant chemotherapy with modestly effective drugs[2]. Unfortunately, due to absence of specific symptoms, a high percentage of patients at the time of diagnosis present a incurable locally advanced or metastatic disease that precludes a successful surgical resection, the only possible curative methods for PDAC, at least for early stages diseases.

Approved in 1996[2], Gemcitabine is the frontline standard chemotherapy used essentially in monotherapy for the treatment of pancreatic cancer with modest results; the innate or acquired resistance to chemotherapy drugs of PDAC remains the major obstacle to its successful control[3]. Early detection of PDAC is difficult if not impossible: benign and malignant lesions share similar clinical presentations and imaging features, making imaging detection of early disease difficult[4], and the majority of available molecular markers possess low specificity[5]. Therefore the median overall survival time is 5 mo and the five years survival is less than 5%, a rate essentially unchanged over the course of the years[6]. Consequently, a deeper understanding the pathobiology of this disease is essential to lead to new targeting strategies.

The route to PDAC

Despite the effort of the scientific community, the etiology of PDAC is still poorly understood undermining the efforts for its prevention and cure. A large number of epidemiological studies suggested that tobacco smoking, alcohol consumptions, obesity, chronic pancreatitis, genetic risk factors and diabetes are risk factors of PDAC[7-9].

Pancreatic adenocarcinoma arises from three precursor lesions: the microscopic pancreatic intraepithelial neoplasia (PanIN) and the macroscopic intraductal papillary mucinous neoplasm and mucinous cystic neoplasm (Figure 1)[10,11].

PanINs are the more frequent preneoplastic precursors of PDAC[12]; they are classified according to the accumulation of architectural, cytologic, and genetic alterations: from PanIN 1 with the appearance of columnar cells with mucin, to PanIN3 (also called carcinoma *in situ*) characterized by a severe cyto-architectural athypia[10].

PDAC is a disease with a well defined progression model of accumulation of genetic alterations ranging from single point mutations to gross chromosomal abnormalities[13-17]. The most frequent and studied alterations determine the activation of epidermal growth factor receptor-KRAS pathway[15]. Although almost all patients posses at least one of these mutations, the late stage disease is characterized by increased genome instability and heterogeneity with an average of 63 genetic alterations, the majority of which are point mutations, grouped in a core set of 12 cellular signaling pathways[18].

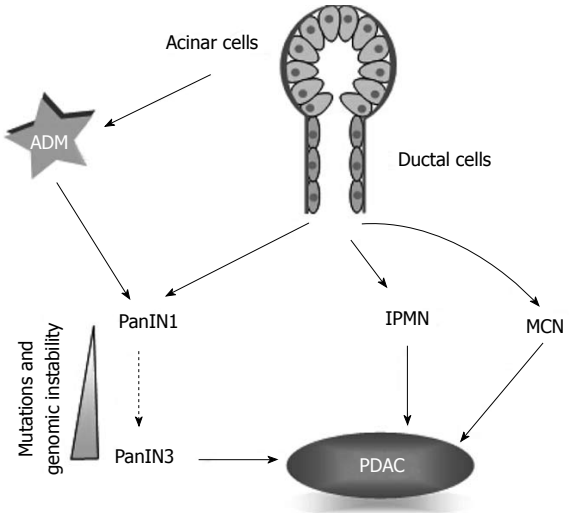

Figure 1 Origin of pancreatic ductal adenocarcinoma. It is widely accepted that pancreatic ductal carcinoma (PDAC) arises from precursor lesions derived from ductal cells; however, recently another model has been proposed where PDAC arises from acinar cells through a process called "acinar to ductal metaplasia" (ADM). PanIN: Intraepithelial neoplasm; IPMN: Intraductal papillary mucinous neoplasm; MCN: Mucinous cystic neoplasm.

Morphological progression from PanIN to PDAC is paralleled by the accumulation of these genetic alterations in a progression model resembling the colon cancer model. Ductal origin of PanIN and PDAC is however questioned and a new model has been proposed where the cancer ductal cells in PanIN lesions originate from metaplastic acinar cells in a process called "acinar to ductal metaplasia" (ADM)[19].

Nuclear receptors: Classification, structural features, and ligands

Nuclear receptors (NRs) are members of a large superfamily of evolutionarily related ligand-regulated DNA-binding transcription factors present in most metazoan[20].

The first NR was only cloned in the '80s by Professor Evans R[21] long after the presence of NR was detected biochemically[22,23]; so far 48 NR has been identified in human by genome sequencing. All NR share characteristic structural features or domains named A to F from the N-terminal to the C-terminal; however, defining features of NR are only the presence of two highly conserved regions: the DNA binding domain (DBD) and the ligand binding domain (LBD), that can function independently[24] and are located in the region C and in the region E of the protein, respectively (Figure 2).

NR are classified into six different subfamilies on homology basis: NR1 (thyroid hormone like), NR2 (HNF4-like), NR3 (estrogen like), NR4 (nerve growth factor IB-like), NR5 (fushi tarazu-F1 like), and NR6 (germ cell nuclear factor like), all originally named from the first member identified. A seventh subclass, NR0, has been introduced to classify two receptors, DAX-1 and SHP, that do not possess the DBD[25] (Table 1, information on ligands obtained from the "nuclear recep-

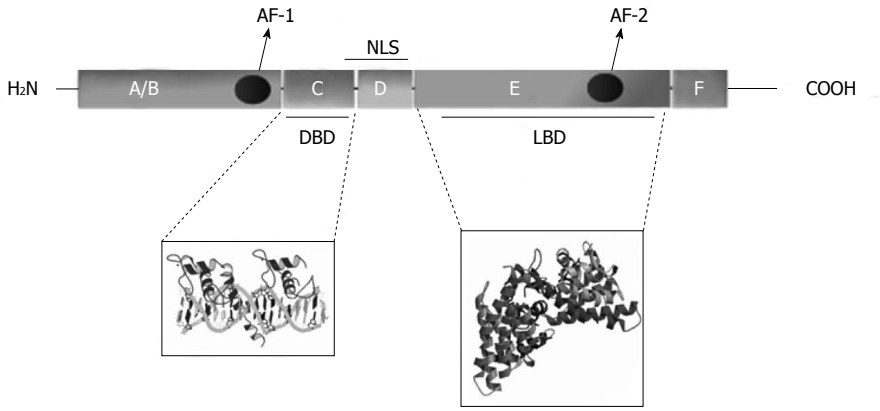

Figure 2 Domains and structural features of a classical nuclear receptor. A typical nuclear receptor (NR) consists of 6 region (A to F); region F may or may not be present. Region D (hinge) contains the nuclear localization signal (NLS); other NLSs may be present in region E. AF-1: Activator function 1; AF-2: Activator function 2.

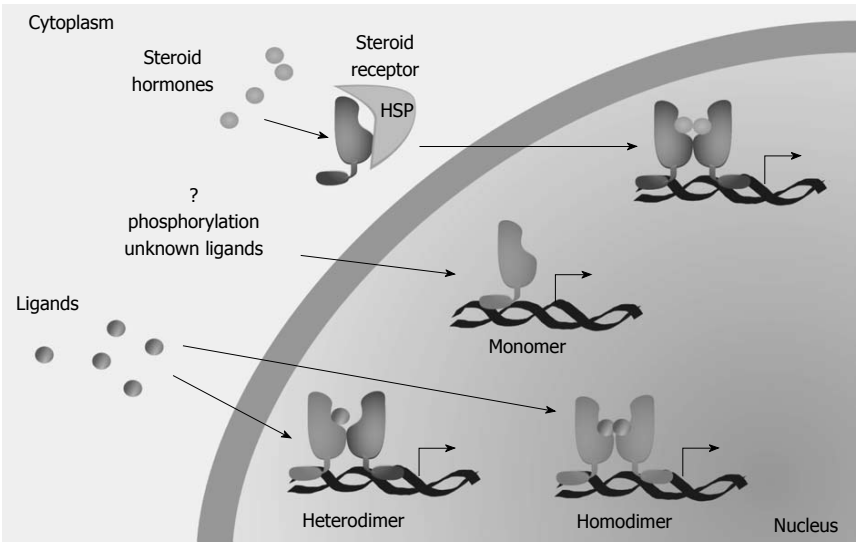

Figure 3 Mechanisms of action of nuclear receptors. Type Ⅰ nuclear receptors (NR) (steroid receptors) are complexed with heat shock proteins (HSP) and maintained in the cytoplasm in the absence of ligands. The other receptors are instead mainly nuclear and the ligands induce hetero- (for type Ⅱ receptors) or homodimerization (for type Ⅲ receptors). Furthermore, a group of receptors (type Ⅳ) whose regulation is poorly known act as monomers.

tor signaling atlas", NURSA, www.nursa.org).

NR ligands are small hydrophobic molecules that bind to the LBD; retinoids, fatty acids, cholesterol, lipophilic hormones and vitamins, as well as some antibiotics, xenobiotics and synthetic drugs are all NR ligands. The ligand binding induces a conformational change that modify the DBD ability to bind specific DNA sequences called response elements. NR act as monomeres, homodimers or heterodimers with the retinoid-X-receptor (RXR) (Figure 3). Upon DNA binding, the final transcriptional activity depends on the presence of co-activator or co-repressor molecules[26].

NR physiologic functions vary from the regulation of metabolism, to growth and development; NR are also implicated with a number of diseases such as cancer. The association of NR with major diseases has transformed these proteins in the most popular and promising drug targets thanks also the properties of their ligands that can easily cross the cell membrane[27].

NR IN PANCREATIC CANCER

NRs are important in the development and homeostasis of the pancreas and their role in PDAC development is the subject of intense study by the scientific community. Here, we will describe the role of a group of these receptors, specifically the peroxisome proliferator activated receptors, the retinoid receptors, the androgen and estrogen receptors, and the orphan NRs.

PEROXISOME PROLIFERATOR ACTIVATED RECEPTORS

The peroxisome proliferator activated receptors (PPARs) belong to the NR1 (thyroid-like) subfamily of NR. Three PPARs are known: PPARα (NR1C1), PPARβ/δ (NR1C2) and PPARγ (NR1C3). PPARγ is the only PPAR with three isoforms with different spatial distribution[28]. They were identified as NR that responded to peroxisome

Table 1 Nomenclature of nuclear receptors

Subfamily official name (class)	NRNC group	Member trivial name	Official name	Abbreviation	Ligand
NR0 (domain-depleted receptors)	B [DAX-like receptors (DAX, SHP)]	Dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1	NR0B1	DAX-1	Orphan
		Short heterodimer partner	NR0B2	SHP	Orphan
NR1 (TR/RAR/PPAR/ VDR-like receptors)	A (thyroid hormone receptors)	Thyroid hormone receptor α	NR1A1	TRα	GC-1, thyroid hormone
	B (retinoic acid receptors)	Thyroid hormone receptor β	NR1A2	TRβ	GC-1, thyroid hormone
		Retinoic acid receptor α	NR1B1	RARα	Am 580, all-trans-Retinoic acid, Arotinoid acid
		Retinoic acid receptor β	NR1B2	RARβ	Am 580, all-trans-Retinoic acid, Arotinoid acid
	C (peroxisome proliferator activated receptors)	Retinoic acid receptor γ	NR1B3	RARγ	Am 580, all-trans-Retinoic acid, Arotinoid acid
		Peroxisome proliferator-activated receptor α	NR1C1	PPARα	GW409544, GW7647, GW6471, Pirinixic acid, Palmitic acid, Leukotriene B4
		Peroxisome proliferator-activated receptor β	NR1C2	PPARβ/δ	Eicosapentaenoic acid, GW0742
		Peroxisome proliferator-activated Receptor γ	NR1C3	PPARγ	Rosiglitazone, GW1929, GW9662, GW409544, GW7647, 15-Deoxy-Δ-12,14-prostaglandin, 15-Deoxy-Δ-12,14-PGJ2
	D [Rev-Erb (NRD, E75)]	Rev-erbα	NR1D1	Rev-erbα	Orphan
	F (RAR-related orphan receptors (ROR, HR3)		NR1D2	Rev-erbβ	Orphan
		Retinoic acid receptor-related orphan receptor α	NR1F1	RORα	Melatonin, CGP 52608
		Retinoic acid receptor-related orphan receptor β	NR1F2	RORβ	Melatonin, CGP 52608
		Retinoic acid receptor-related orphan receptor γ	NR1F3	RORγ	Melatonin,CGP52608
		Liver X receptor β	NR1H2	LXRβ	GW3969, T0901317, oxysterols
	H (ecdysone-like receptors)	Liver X receptor α	NR1H3	LXRα	GW3969, T0901317, oxysterols
		Farnesoid X receptor α	NR1H4	FXRα	GW4064, bile acid chenodeoxycholic acid
	I (vitamin D3-like receptors)	Vitamin D3 receptor	NR1I1	VDR	1,25-dihydroxyvitamin D3
		Pregnane X receptor	NR1I2	PXR	Hyperforin, SR12813, rifampicin, pregnenolone carbonitrile, T0901317, 24(S),25-epoxycholesterol, butamben
		Constitutive androstane receptor	NR1I3	CAR	Androstanol, CITCO, phenobarbital, ATE
NR2 (HNF4/RXR/ TLL/COUP-like receptors)	A (hepatocyte nuclear factor 4)	Hepatocyte nuclear factor 4 α	NR2A1	HNF4α	Palmitoyl coenzyme A
		Hepatocyte nuclear factor 4 γ	NR2A2	HNF4γ	Orphan
	B (retinoid X receptors)	Retinoid x receptor α	NR2B1	RXRα	LGD 100268, GW0791, 9-retinoic acid
		Retinoid x receptor β	NR2B2	RXRβ	LGD 100268
		Retinoid x receptor γ	NR2B3	RXRγ	LGD 100268
		Testis receptor	NR2C1	TR2	Orphan
	E (tailless-like receptors)		NR2C2	TR4	Orphan
		Tailless	NR2E2	TLL	Orphan
		Photoreceptor-specific nuclear receptor	NR2E3	PNR	Orphan

	F [COUP-TF-like receptors (COUP-TF, SVP, EAR2)]	Chicken ovalbumin upstream promoter transcription factor Ⅰ	NR2F1	COUP-TFⅠ	Orphan
		Chicken ovalbumin upstream promoter transcription factor Ⅱ	NR2F2	COUP-TFⅡ	Orphan
		ErbA2-related gene-2	NR2F6	EAR2	Orphan
NR3 (ER/ERR/GR/MR/PR/AR)	A	Estrogen receptor	NR3A1	Erα	Fulvestrant, 17β-estradiol, 4-hydroxytamoxifen, Raloxifene
			NR3A2	Erβ	Fulvestrant, 17β-estradiol, 4-hydroxytamoxifen, Raloxifene
	B	Estrogen receptor-related receptor	NR3B1	ERRα	Orphan
			NR3B2	ERRβ	GSK4716, Diethylstilbestrol
			NR3B3	ERRγ	GSK4716, 4-hydroxytamoxifen
	C	Glucocorticoid receptor	NR3C1	GR	Dexamethasone, hydrocortisone, RU486
		Mineralocorticoid receptor	NR3C2	MR	Spironolactone, aldosterone, RU486
		Progesteron receptor	NR3C3	PR	R5020, progesterone, RU486
		Androgen receptor	NR3C4	AR	Dihydrotestosterone, RU486, Bicalutamide, R1881
NR4 (NGFIB-like receptors)	A [nerve growth factor IB-like receptors (NGFIB, NURR, NOR, HR38, CNR-8)]	Growth factor-inducible immediate early gene Nur77	NR4A1	Nur77	Orphan
		Nur-related protein 1	NR4A2	NURR1	Orphan
		Neuron-derived orphan receptor 1	NR4A3	NOR1	Orphan
NR5 (FTZ-F1/SF1-like receptors)	A [fushi tarazu F1-like receptors (SF1, FTF, FTZ-F1)]	Steroidogenic factor-1/ELP	NR5A1	SF1	Orphan
		Liver receptor homolog 1	NR5A2	LRH-1	Orphan
NR6 (GCNF)	A (germ cell nuclear factor)	Germ cell nuclear factor 1	NR6A1	GCNF1	Orphan

PPAR: Peroxisome proliferator-activated receptor; PDAC: Pancreatic ductal carcinoma; TZD: Thiazolidinediones; IFN: Interferon; RAR: Retinoic acid receptor; RXR: Retinoid X receptor; AR: Androgen receptor; ER: Estrogen receptor; LHR-1: Liver receptor homologue-1 receptor; COUP-TFⅡ: Chicken ovalbumin upstream promoter transcription factor Ⅱ.

proliferators, heterogenous chemicals that increase the number of peroxisomes (making them "proliferate") in hepatocytes[29]. Natural ligands for PPARs are free fatty acids and PPARγ is also activated by 15-Deoxy-delta (12,14)-prostaglandin J(02)(15d-PGJ2)[30]. Some other PPAR ligands are the PPARα agonists hypolipidemic drugs fibrates and leukotriene B4 (LB4), the PPARγ specific agonist and antidiabetic drugs thiazolidinediones (TZD), and the PPARβ/δ specific agonist GW501516[31].

PPARs show a different expression pattern, and PPARβ is the most widely expressed[32]; they act as heterodimers with RXR and regulate complex gene networks especially in energy homeostasis and inflammation[28,33,34]. Furthermore they are involved in a spectrum of disease such as alcoholic liver disease and may mediates oxidative stress response[28,30,32,34].

PPARα

PPARα sustained activation leads to the development of cancers in the liver, testis and pancreas in rodents[35,36]. Moreover, PDX-1, an oncogene for pancreatic cancer that is overexpressed in PDAC[37], is a PPARα -dependent gene and its expression is downregulated by MK886, a specific PPARα antagonist[38]. However PPARα -dependent carcinogenesis has been recently questioned[39].

PPARβ/δ

Recently it has been reported that PPAR signaling, especially PPARβ/δ, is reduced in pancreatic cancer relapse, compared to primitive cancer[40], but PPARβ/δ has been suggested to be a critical component of the angiogenetic switch in pancreatic cancer[41,42]. Abdollahi[42] showed that the expression of PPARβ/δ detected by immunohistochemistry in human pancreatic specimens high-density tissue microarrays correlated with tumor staging; indeed PPARβ/δ staining intensity increased from normal pancreas to chronic pancreatitis, pancreatic cancer and metastasis and the up-regulation of PPARβ/δ is actually more enhanced in the tumor vasculature and in the tumor stroma[42]. High expression of PPARβ/δ in tumor is also confirmed by a recent paper[31]. Elevated PPARβ/δ expression levels are also highly correlated with advanced stages of tumor progression and with increased risk for tumor recurrence or distant metastasis[42]

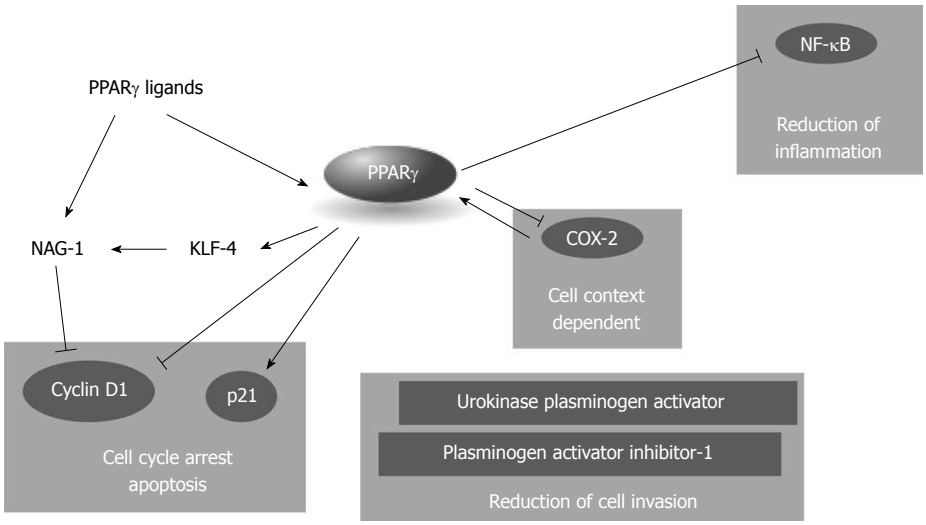

Figure 4 Peroxisome proliferator activated receptors and pancreatic ductal carcinoma. Peroxisome proliferator activated receptor (PPAR)γ acts as a tumor inhibitor at multiple levels, blocking cell cycle progression, inflammation, and cell invasion. NAG-1: Non steroidal anti-inflammatory drug-activated gene-1; Cox-2: Ciclooxigenase-2; NF-κB: Nuclear factor-κB.

and PPARβ/δ has been proposed as a "central hub" in tumor angiogenesis given that tumor growth and angiogenesis were greatly reduced when tumor cells were implanted in PPARβ/δ null mice[42].

PPARβ/δ is also expressed in human pancreatic cancer cells and its activation regulates the metallo-protease matrix metalloproteinases (MMP)-9, decreasing cancer cells ability to transverse the basement membrane[31]. PPARβ/δ activation reduces the tumor necrosis factor (TNF)α-induced expression of various genes implicated in metastasis increasing the availability of the transcriptional repressor B-cell lymphoma (BCL)-6. BCL-6 is bound to PPARβ/δ and is released after GW501516 treatment resulting in decreased MMP-9 expression. These results suggest that increased expression of PPARβ/δ regulates pancreatic cancer cell invasion sequestering BCL-6 and hence inducing MMP-9 mediated invasion[31].

PPARγ

PPARγ is not only implicated in adipocyte differentiation, lipid accumulation, and glucose homeostasis but it is also an important regulator of inflammation *via* the inhibition of, or the interference with, proinflammatory signalings such as signal transducers and activators of transcription (STATs), nuclear factor-κB (NF-κB), and activator protein-1 (AP-1)[28,30].

PPARγ is expressed in primary PDAC[43-46] and strongly correlates with a more advanced clinical stage; PPARγ staining is also associated with shorter overall survival and its has proved to be an independent prognostic factor in uni- and multi-variates analysis[43,45]. However, whereas Kristiansen *et al*[45] found a strong overexpression of PPARγ by expression profiling in 19 microdissected carcinoma compared to 14 ductal epithelia, Pazienza *et al*[44] did not find significant alterations in the expression levels of PPARγ in pancreatic cancer. A possible explanation of this discrepancy may be that is the expression *"per se"* of PPARγ, and not its levels, important in pancreatic cancer progression. Interestingly, a large number of studies have demonstrated that TZD reduce the risk of PDAC[9].

A genetic association PPARγ/PDAC has been tested by two different groups[47,48] analyzing the expression of the single nucleotide polymorphisms (SNP) Pro12Ala that has been associated with reduced risk of diabetes and some cancers[47]. Fesinmeyer *et al*[48] demonstrated that this SNP is associated with increased risk of PDAC in a high-risk sample of smokers randomized to high-dose vitamin A; however, two years later, a similar study in obese and diabetic patients demonstrated a protective role of the SNP, prompting the need of further studies[47].

In vitro, the role of PPARγ is controversial but it is generally accepted that the receptor acts as a tumor inhibitor at multiple levels (Figure 4). PPARγ is expressed in human pancreatic cancer cell lines[46,49-55] and its expression follows a circadian rhythmicity with a period of 24 h that could potentially influence the cell phenotype and the human disease behavior[55]. Agonists of PPARγ such as TZD, its natural ligand 15d-PGJ2, or 1,1-Bis(3-indolyl)-1-(p-substituted phenyl)methanes (C-DIMs), induce cell cycle arrest in G1, apoptosis and ductal differentiation in pancreatic cancer cells[46,53,54,56-58]. However some of the effects associated with PPARγ activation are instead receptor independent: this is especially true for TZD agonists whose receptor independent effects have been widely described[51,52,59-69].

Cell cycle arrest is associated to decreased PPARγ-dependent expression of cyclin D1[53,54,57,58] whereas the reported induction of p21 might be PPARγ-dependent or PPARγ-independent[52,54]. Deletion analysis of the p21 promoter indicates that PPARγ-dependent activation of p21 requires GC-rich sites in the proximal region of the promoter[54]. It is worth to note that some agonists of PPARγ[61] may induce a PPARγ independent down-

regulation of cyclin D1, due to induction of non steroidal anti-inflammatory drug-activated gene-1 (NAG-1). NAG-1 is a member of the TGF-β superfamily involved in tumor progression acting as a pro-apoptotic gene. Interestingly, it has been reported that NAG-1 expression is positively regulated by PPARγ: MCC-555, a PPARγ agonist, induces the expression of the transcription factor KLF-4 in PPARγ-dependent manner who subsequently enhances the NAG-1 promoter activity[51,52]. PPARγ agonists reduce the invasive capacity of PDAC cells with a PPARγ dependent mechanism[50]. The PPARγ ligands 15d-PGJ2 and ciglitazone attenuate pancreatic cancer cell invasion increasing plasminogen activator inhibitor-1 and decreasing urokinase plasminogen activator levels resulting in the reduction of total urokinase activity in pancreatic cancer cells[50]. Interestingly, the PPARγ antagonist T0070907 suppresses pancreatic cancer cell motility by altering the localization of p120 catenin and by suppressing the activity of the Ras-homologous GTPases Rac1 and Cdc42[49].

The effects of PPARγ activation or inhibition by specific molecules sinergize or interact with other pathways or other NR activations[53,56,62-64]. Combination of recombinant interferon-β (IFN-β) and the PPARγ agonist troglitazone induces a synergistic effect on the growth inhibition of pancreatic cancer cells, through the counteraction of the IFN-β-induced activation of STAT-3, MAPK and AKT and the increase in the binding of both STAT-1 related complexes and PPARγ with specific DNA responsive elements. The combination induces also an increase in autophagy and a decrease in anti-autophagic bcl-2/beclin-1 complex formation, mediated by the inactivation of the AKT/mTOR-dependent pathway[64]. PPARγ form mandatory heterodimers with RXRα and its activity is maximal in the presence of RXRα agonists; it is not surprising then that co-treatment with PPARγ and RXRα agonists exacerbates the effects of PPARγ increasing the inhibition of cell growth[53,62,63]. Synergistic effects on growth inhibition are also visible when inhibitors of ciclooxigenase-2 (Cox-2) and PPARγ agonists are used in combination[56,65]. Cox-2 is an inducible ciclooxygenase that contributes to the metabolism of arachidonic acid forming prostaglandin H2, a precursor of 15d-PGJ2[66]. Cox-2 is a downtarget of PPARγ and its expression may be either induced or repressed by the NR, depending on the cell context[30]. However, selective Cox-2 inhibitors have opposite effects in pancreatic cancer depending on Cox-2 expression: in high Cox-2-expressing cells the inhibitors reduced tumor growth; conversely, in Cox-2 negative or low expressing cancer cells the inhibitors, at very high concentrations, enhance tumor progression increasing intratumoral VEGF and tumor angiogenesis in a PPARγ-dependent way[65].

PPARγ is known to reduce tumor growth in mice *in vivo*[49,62,65,67] and its activation increases the gemcitabine mediated tumor suppression[68]. Tumor growth inhibition mediated by PPARγ may be due to reduced inflammation and increased activation of anti-inflammatory genes[30,67]. Genetic deletion of Ikk2, a component of the canonical NF-κB signaling pathway, in the Kras(G12D)Pdx1-cre mouse model of pancreatic cancer, substantially delays pancreatic oncogenesis and results in downregulation of the classical Notch target genes Hes1 and Hey1[67]; in the same model TNF-α stimulation resulted in increased Hes1 expression and consequent suppression of PPARγ expression facilitating the formation of a inflammatory pro-tumoral enviroment; induction of PPARγ instead may block NF-κB induced processes[30] reducing or delaying tumor formation[67].

Despite all these intriguing discoveries on PPARγ role in PDAC, clinical application of PPARγ modulation has recently suffered yet another failure when a new oral anticancer agent with LB4 antagonist and PPARγ agonist properties[69,70], the LY29311, did not demonstrate any benefit in association with gemcitabine in unpretreated patients with advanced PDAC[69].

RETINOIC AND RETINOID RECEPTORS

Retinoic acid receptors (RARs) and RXRs are NRs transcription factors that bind retinoids, natural and synthetic molecules structurally and/or functionally related to vitamin A, and regulate cell differentiation, proliferation, and survival[25,71,72]. A list of retinoids with biological functions comprises, but is not limited to, all trans retinoic acid (atRA), 9-*cis*-retinoic acid (9-*cis*-RA), 11-*cis*-RA, 13-*cis*-RA, being the atRA the predominant physiological form; retinoids that specifically bind to RXR are called rexinoids and have been effective in cancer treatment. RARs can be activated by both atRA and 9-*cis* RA, while RXRs are exclusively activated by 9-*cis*-RA, initially identified as a *bona fide* RXR ligand *in vitro*[71], but never detected *in vivo*[73]. Other RXR natural ligands have been identified *in vivo* but they are not RXR specific ligands[74-76].

RARs and RXRs are each encoded by three different genes that give rise to the -α, -β, -γ isoforms of RXR and RAR, each presenting transcription variants, and characterized by different spatial distribution[77,78]. RXRs were identified as cofactor for efficient binding of RAR to its DNA response elements[79], but unique among the NRs, RXR play a modulatory role along multiple pathways forming mandatory dimers with thyroid hormone receptor, PPAR, vitamin D receptor (VDR), RAR, Nur77, *etc.*[25,71]; in these heterodimers RXR may function as an active partner (such in the case of PPARγ:RXR dimers) meaning that the dimers respond to 9-*cis*-RA, or as silent partner and the dimers do not respond to RA.

Due to their regulatory potential, these NRs are major drug targets for a number of pathologies, including cancer and metabolic diseases.

RAR and RXR receptors are expressed during pancreatic organogenesis and are essential for ductal differentiation[80,81]. Retinoid receptors are more expressed in the exocrine compartment, usually during late gestation, with a strong lineage specificity. Exogenous 9-cis-

RA induces predominantly ducts instead of acini, plus more mature endocrine architecture, whereas exogenous atRA induces predominantly acini instead of ducts, with no apparent endocrine effect[80]. RAR-selective agonists mimicked the acinar suppressive effect of 9-*cis*-RA, suggesting that RAR-RXR heterodimers are critical to ductal differentiation; however, retinoids do not regulate exocrine lineage selection cell-autonomously but epithelial-mesenchymal interactions are mandatory given that 9-*cis* -RA does not induce ductal differentiation in the absence of mesenchyme and requires the presence of laminin-1[81]. The ability to restore a more differentiated phenotype and to regulates ductal differentiation may explain the effects of retinoids.

Expression of RXR and RAR has been described in pancreatic cancer cell lines and PDAC[42,53,62,63,82-88] but their biological and clinical significance is not clear: in most cases RXR and RAR receptors apparently act as tumor suppressors in PDAC cancer both *in vivo* and *in vitro*[43,53,62,63,84-88], inducing arrest in cell proliferation and differentiation although results suggestive of a pro-oncogenic role are also reported[89-91].

A differential expression of RAR-α, -β, and -γ, and RXRα was detected in histological sections of human PDAC and their adjacent normal tissues. Whereas all four receptors were detected in adjacent normal pancreatic tissue specimens, RARβ mRNA transcripts were detected in only 67% of the malignant tissues and when expressed, the level of expression was significantly lower than that of the corresponding adjacent normal tissues, especially in moderately- and poorly-differentiated cancers[92]; these results are in agreement with previous papers showing that RARβ expression is lost during PDAC malignant transformation[84]. The mechanisms at the basis of RARβ mRNA downexpression are not known but it is worth noting that in pancreatic endocrine neoplams the NR promoter is often hypermethylated[93,94], suggesting that in certain pancreatic carcinomas the reduction or loss of RARβ expression by epigenetic mechanisms might be associated with the development or progression of tumors[92]; interestingly one missense mutation in RARβ has also been identified in PDAC[18]. The anti-tumoral role of RARβ is confirmed by its overexpression in DAN-G pancreatic cancer cells that results in induction of differentiation and inhibition of proliferation *in vivo* and *in vitro*[84].

Immunohistochemical evaluation of PPARγ and RXRα protein expression in 65 PDAC patients statistically analyzed in relation to clinicopathological characteristics, tumor proliferative capacity, and patients' survival showed that 75% of patients tested positive for PPARγ and 85% stained positive for RXRα. Interestingly, RXRα positivity was significantly associated with tumor proliferative capacity and PPARγ positivity but RXRα failed to predict patients' survival[43].

In vitro, RXR and RAR involvement in PDAC has been usually tested by means of specific agonists or antagonists. Retinoids may be useful agents for the treatment of pancreatic cancer; however, RAR-selective retinoids produce unwanted side effects. In contrast, RXR-selective retinoids produce fewer side effects. The reported results indicate that these receptors often possess antiproliferative and pro-differentiative effects whereas reports on induction of apoptosis are mixed[82,83,88].

In 13 cell lines established from patients who underwent surgery for PDAC Albrechtsson *et al*[86] detected the expression of the RAR and RXR subtypes and evaluated the effect of atRA and 9-*cis*-RA on cell proliferation. They demonstrated that RARα, β and RXRβ were expressed in most of the cell line. RXRγ was expressed in about half of them and RARγ in only one whereas the RXRα receptor was expressed in all cell lines. Incubation of the cells with atRA or 9-*cis*-RA reduced cell proliferation, although only about half of the cell lines responded to the latter[86]. These results partially contradict a previous paper that showed that pancreatic cancer cell lines *in vitro* responded to 9-*cis*-RA but not atRA at clinically relevant concentrations[87]. Moreover, as previously reported[53], 9-*cis*-RA acts additively with the TZD Troglitazone blocking the cells in G1 phase through reduction of cyclin D1 levels.

The RXR-selective retinoid, AGN194204 inhibits the proliferation of pancreatic cancer cells more efficiently than RAR-selective retinoids, but does not increase the apoptosis, whereas other retinoids are also able to induce apoptosis[82]. Block of cell proliferation in these cells is associated with reduced cyclin E and cyclin dependent kinase 6 levels, an effect reversed by the RXR antagonist AGN195393 but not by RAR antagonist AGN193109. Treatment of MIAPaCa-2 cells with AGN194204 and cytotoxic agents such as gemcitabine, 5-fluorouracil, or IFNγ resulted in an additive but not synergistic reduction in cell number[95]. Interestingly, the retinoid-related ligand AGN193198 reduces BxPC-3, MIAPaCa-2 and AsPC-1 cell proliferation (blocking the cell in the S phase) more efficiently than high-affinity RAR- or RXR-selective retinoids and induces apotosis[88]; however the compound does not activate transcription from RAR or RXR response elements and its effects on cell survival are not reversed by treatment with RAR- or RXR receptor-selective antagonists. These results suggest that AGN193198 (but we may not exclude also other retinoids) might act independently of the classical retinoid receptors.

Treatment of pancreatic cancer cells with 9-*cis*-RA induces apoptosis lowering the ratio Bcl2/Bax2 and requires the presence of RARγ[83]. Interestingly, 9-*cis*-RA acting on RXRα may induce the nuclear export of Nur77[96,97], facilitating its interaction with Bcl2 and hence increasing the apoptosis (see later for details).

Retinoids possess the ability to induce pancreatic cancer differentiation *in vitro*[82,89,98]. The differentiation phenotype changes are associated with increase in aerobic metabolism, expression of mucins, synthesis and secretion of TGF-β, and reduction of EGF receptor expression[82]. This differentiation effects are dependent

on TGF-β, because co-treatment with atRA and a pan-TGF-β neutralizing antibody abolishes the anti-proliferative and pro-differentiative effect of the retinoid and reduces MUC4 expression[82,89]. As previously described, ADM might play an important role in PDAC development and DSL-6A/C1 cells, who expresses RAR-α and -β and RXRα, represent an *in vitro* model of this carcinogenic sequence[98]. Treatment of DSL-6A/C1 cells with retinoids results in a time- and dose-dependent inhibition of cell growth, paralleled by a retinoid-mediated transactivation of a pTK:betaRAREx2-luciferase reporter; growth inhibition is reverted by the RARα specific antagonist Ro 41-5253, suggesting that the RARα might influence ADM[98].

Regulation of expression of mucins (MUCs) by retinoids however raises questions regarding the response of pancreatic tumor cell *in vivo*. RAR and RXR receptors have been reported to influence the expression of MUC4 and MUC17[89-91] and RXR:VDR response elements are present in the promoter region of *MUC17* gene[90]. Both mucins are associated to the progression of pancreatic cancer: MUC17 is linked to the presence of lymph node metastasis[99] and MUC4 expression increases during progression of PDAC from PanIN1 to PanIN3, and it is highly expressed in invasive adenocarcinomas[100-102]. The expression of *MUC17* gene is regulated by a 1146-bp DNA fragment upstream of MUC17 that contains GATA, NF-κB, Cdx-2 and RXR:VDR response elements, but no data are available on the role of the latter. Instead, retinoids directly regulates the expression of MUC4[91], sinergistically with IFNγ and dependently of TGF-β. Interestingly, IFNγ has been shown to possess antitumor activity and it is well known that TGF-β possess tumor suppressive and oncogenic activities[103]. At early stages TGF-β acts as a tumor suppressor, whereas at later stages tumor cells become resistant to its antiproliferative effects but continue to secrete high quantity of the factor. Indeed, pancreatic tumors overexpress all the three TGF isoforms and this correlates with decreased patient survival[104] and induction of epithelial to mesenchymal transition (EMT). On the other hand, although IFNγ is antiproliferative *in vitro* against pancreatic cancer cells, the temporal aspect of this process has never been studied. Indeed, the expression of MUC4 does not require the continuing presence of IFNγ or RA which instead are required for the priming of MUC4 expression[91]. From a pathological point of view, aberrant expression of mucins on the surface of PDAC cells may provide protection against the host's activated immune system while conferring antiadhesive properties upon the cells and hence favoring the EMT-mediated metastatization, casting a shadow on the use of retinoids *in vivo*. Nonetheless, retinoids have been used in phase Ⅱ clinical trials in the past[105-107] with mostly disappointing results. In 1998, basing on promising *in vitro* results, one trial in which patients with advanced PDAC were treated with 13-*cis*-RA and IFNα resulted in prolonged stable disease in two third of the patients[105]; this however contradict a 1995 phase Ⅱ trial where the same therapeutic regimen did not improve patients condition[107]. Furthermore, the combination of 13-*cis*-RA with gemcitabine in a more recent phase Ⅱ clinical trial, although well tolerated, did not determine an improvement in the response[106]. PDAC resistance to retinoid treatment might be dependent on the relative intracellular expression of the retinoids-binding proteins fatty acid-binding protein 5 (FABP5) and cellular retinoic acid-binding protein 2 (CRABP2)[108] that were shown to be critical for either antisurvival (CRABP2) or prosurvival (FABP5) effects of retinoic acid[109].

ANDROGEN AND ESTROGEN RECEPTORS

Androgen receptor (AR, NR3C4) and estrogen receptors (ER)-α and -β (NR3A1 and NR3A2) belong to the steroid receptor subfamily (NR3). These NR regulate multiple physiological processes including sexual development and are implicated in multiple cancers[110-113].

Their involvement in PDAC has long been suggested by the evidence that pancreatic cancer shows an apparent hormonal imbalance in the incidence with a male to female ratio ranging from 1.25-1.75:1[114,115], that approach the 1:1 ratio with advancing age[116] (for recent reviews see[117,118]).

In the early '90s, the presence of AR in PDAC was questioned, but recent papers clearly demonstrated that pancreatic cancer cells express detectable levels of AR[117,119,120]. *In vitro*, PDAC cancer cells variably respond to the treatment with the agonist testosterone, showing a modest increase in cell proliferation[119,121]. Flutamide, an AR blocker used for the treatment of prostate cancer, induces a reduction in cell proliferation that does not however correlate with AR expression levels[119]. Furthermore, flutamide treatment does not alter the cell response to gemcitabine *in vitro* and *in vivo*[119]. AR activity is modulated by IL-6, an inflammatory cytokine overexpressed in pancreatic cancer[120,122]. IL-6 enhances STAT3 and MAPK pathways that in turn increase the AR transcriptional activity; IL-6 also enhances pancreatic cancer cell migration in the presence of AR, an effect blocked by the silencing of the receptor[120] (Figure 5A). In a double-blind placebo-controlled trial, flutamide doubled the survival duration when administered in a dosage of 250 mg three times daily[123]. This excellent result has not been confirmed by other small phase Ⅱ trials where flutamide was used in monotherapy or in combination with gemcitabine[124,125]. The lack of AR response in PDAC patients suggests that the tumor cells, although expressing the AR, are in a hormone-refractory proliferative status, as observed in prostate cancer[119].

The role of ER in PDAC is controversial: although several papers described the presence of ER (usually ERα) in primary PDAC, other reports did not detect the receptors at all[118,126,127] and the antiestrogen tamoxifen has been used in clinical trials with no benefit[118,128]. Expression of both ERα and -β has been described in

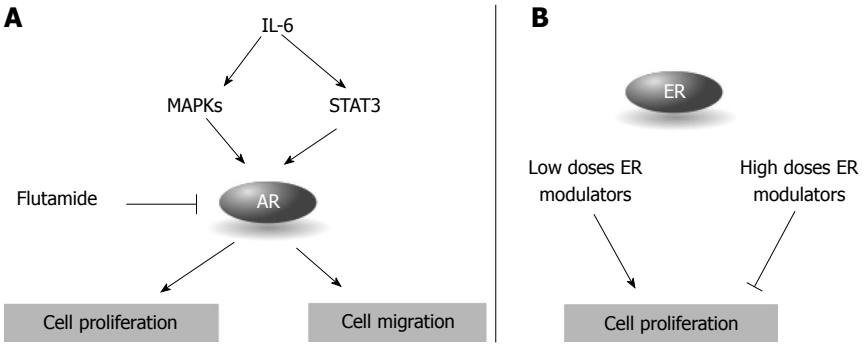

Figure 5 Steroid receptors in pancreatic ductal carcinoma. A: Androgen receptor (AR) regulates cell proliferation and migration and it is activated by the inflammatory cytokine interleukin-6 (IL-6); B: The effect of estrogen receptors (ER) on cell proliferation depends on the concentrations of the ER modulators.

pancreatic cancer cells[129] and a recent proteomic validation study in formalin-fixed paraffin-embedded tissues identified several proteins tightly associated through ERα[130]. *In vitro*, PDAC cells respond to the treatment with estrogens modulating agents: lower concentrations usually induce cell proliferation whereas high concentrations arrest cell proliferation (Figure 5B)[129]. The response seems dependent on the expression of ERα and ERβ, specifically to their ratio. ERα and ERβ share an almost perfect homology in the LBD that allows both of them to bind estrogen; other domains are instead less conserved with the most divergent region being the A/B domain, characterized by the absence of the activator function-1 in the ERβ. Analyzing the expression of the two estrogen receptors Iwao *et al*[129] found that pancreatic cancers showed significantly lower ERα mRNA levels than ER-negative breast cancers while ERβ mRNA levels (that were higher in ER-negative than ER-positive breast cancers) were significantly higher than ER-negative breast cancers; in seven out of eight pancreatic cancer cell lines ERβ outweighs ERα and cells with lower ERα/ERβ ratio tend to have higher responsiveness[128], suggesting that ERβ may play a more important role in PDAC[118,128]. Interestingly, phytoestrogens such as genistein block pancreatic cancer growth *in vitro* and show higher affinity for ERβ.

OTHER NRS: ORPHAN NRS IN THE SPOTLIGHT

Roughly half of the 48 human NR are classified as orphan NRs. The orphan NRs form a specific subgroup of the NR proteins characterized by different functional and evolutionary origin; orphan NR are distributed along the all the six NR subfamilies. These proteins have in common only the term "orphan receptor" conied few decades ago to describe, by definition, gene products that appear to belong to the nuclear receptor family on the basis of sequence identity but for whom no ligands is known[131,132]. Orphan receptors diverge also at the structural levels with various examples of members without all the classical features of nuclear receptor, such as the LBD or the DBD[132]. The discovery of the orphan receptors drastically changed endocrinology introducing the "reverse endocrinology" approach where orphan receptors are used to identify new hormones and their associated biology, conversely to traditional endocrinology that identified hormones starting from their physiological or pathological effect[131].

Although the term "receptor" implies the existence of a natural ligand, this assumption is debated and not necessarily true for at least some of the orphan NRs[25]. In time, the number of orphan receptors has diminished due to the discovery of natural ligands for some of them that have then become "adopted", such in the case of PPARγ or FXR for example[25].

Here we will discuss recent findings on orphan receptors, specifically the NR4As, the liver receptor homologue-1 receptor (LRH-1) and the chicken ovalbumin upstream promoter transcription factor Ⅱ (COUP-TFⅡ) discussing their role in PDAC.

Nur77: A "two faces" action in pancreatic cancer

The orphan NR subfamily 4 subgroup A (NR4A) is comprised by three members: NR4A1 (also known as Nur77, testicular receptor TR3, or nerve growth factor 1b NGFI-B), NRA42 (Nurr-related factor 1) and NR4A3 (neuron-derived orphan receptor1, Nor-1)[25,133]. The first member of NR4A family was identified by differential hybridization in the rat pheochromocytoma cell line PC-12 cell as encoded by an immediate early gene (*i.e.*, a gene that is rapidly and transiently transcribed in response to stimuli) induced by the nerve growth factor[134]. As in the case of other NRs, the NR4A members show common features of a classic nuclear receptor and an high degree of sequence homology specifically in the DBD and LBD regions, where homology may be as high as 95% (Figure 6).

All three members are localized in the nucleus due to the presence of a nuclear localization signal in the DBD (three signals in the case of NR4A1). The three members of the family show a different and often overlapping expression in adult tissues, being Nur77 more abundantly and broadly expressed[133]. NR4A receptors might act as monomere or as homo- hetero-dimers with different affinity to DNA response elements; dimers show

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NR4A2    HVPMNPEPAGSHHVVDGQTFAVPNPIRKPAS-MGFPGLQIG---HASQLLDT--QVPSPP   252
NR4A3    SLPLGAAAAAGSQAAALESHPYGLPLAKRAAPLAFPPLGLTPSPTASSLLGESPSLPSPP   281
NR4A1    QSPLKLFPSQATHQLG-EGESYSMPTAFPGLAPTSPHLEGS------GILDTPVTSTKAR   256
           *:   .: . :    :  .   *    .     * *         :*.     ...

NR4A2    SRGSPSNEGLCAVCGDNAACQHYGVRTCEGCKGFFKRTVQKNAKYVCLANKNCPVDKRRR   312
NR4A3    SRSSSSGEGTCAVCGDNAACQHYGVRTCEGCKGFFKRTVQKNAKYVCLANKNCPVDKRRR   341
NR4A1    SGAPGGSEGRCAVCGDNASCQHYGVRTCEGCKGFFKRTVQKNAKYICLANKDCPVDKRRR   316
         * .. ..** ********:************************:*****:********

NR4A2    NRCQYCRFQKCLAVGMVKEVVRTDSLKGRRGRLPSKPKS----PQEPSPPSPPVSLISA   367
NR4A3    NRCQYCRFQKCLSVGMVKEVVRTDSLKGRRGRLPSKPKSPLQQEPSQPSPPSPPICMMNA   401
NR4A1    NRCQFCRFQKCLAVGMVKEVVRTDSLKGRRGRLPSKPKQ--------PPDAFPANLLTS   367
         ****:*******:**************************.          .* : *  ::.:

NR4A2    LVRAHVDSNPAMTSLDYSRFQANPDYQMSGDDTQHIQQFYDLLTGSMEIIRGWAEKIPGF   427
NR4A3    LVRALTDSTP--RDLDYSRYCP-TDQAAAGTDAEHVQQFYNLLTASIDVSRSWAEKIPGF   458
NR4A1    LVRAHLDSGPSTAKLDYSKFQELVLPHFGKEDAGDVQQFYDLLSGSLEVIRKWAEKIPGF   427
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Figure 6 Clustal sequence alignment of human NR4As. Shaded sequences correspond to the DNA binding domain where homology among NR4A1-3 is very high.

stronger activity over monomers[133]. The crystallographic analysis of NR4A receptors suggests that the members of this subfamily are constitutively activated: their LBD is almost completely occupied by bulky aminoacid side chains conferring a 3D structural conformation similar to that of agonist-bound receptors[132,133]. Consequently, unlike others NR, the activity of NR4As is not regulated by stimuli through a ligand binding but instead by modulation of their expression or *via* post-translational modifications[132,133]. Expression of these receptors is induced by a range of stimuli, including stimuli associated with metabolic functions, such as: fatty acid, growth factors, prostaglandines, membrane depolarization, cold, glucose, cholesterol, TZD and hormones[135]. Other hormones may regulate NR4A transcriptional activity interacting with the NR4 heterodimers, such 9-*cis*-RA on NR4A: RXR dimers, and new molecules have been identified acting as agonists or antagonists[96,97,136].

The involvement of NR4A2 in pancreatic cancer *in vitro* has been reported by two papers[133,136]. NR4A2 is highly expressed in many cancer cell lines including Panc1 and Panc28 pancreatic cancer cells. Structure-dependent activation of NR4A2 by a series of C-DIM analogs, especially a p-bromophenyl analog (DIM-C-pPhBr), alters the expression of NR4A2 target genes; among the altered genes the drug determines a NR4A2-dependent repression of neuropilin (NP)-2 in both cell lines, whereas it induces the expression of NP-1 in PANC28 but not PANC1[136]. NPs are important in the progression of pancreatic cancer and are currently seen as potential target for PDAC treatment[137] and it is conceivable that differential expression of these molecules by NR4A2 might be important for PDAC development. Moreover, in PC3 cells NR4A2 acts as a pro-survival antiapoptotic factor[133]: silencing of the receptor greatly reduced the anchorage independent growth, with minimal effect in anchorage-dependent growth, largely due to increase anoikis; NR4A2 silencing also impaired the formation of tumors in nude mice[133].

More data link NR4A1 to pancreatic cancer, depicting a complex mechanism of action where Nur77 acts as having "two faces", being pro-survival and anti- and pro-apoptotic at the same time.

NR4A1 is expressed as a nuclear protein in pancreatic cancer cells[136,138-142] and was found to be overexpressed in PDAC tissues primarily in the nucleus, whereas 83% of non-tumor pancreatic tissues did not express it[142].

c-DIMs are a class of molecules that activate PPARγ and might act on NR4A receptors[136,138,139]; a c-DIM, specifically the 1,1-*Bis*(3'-indolyl)-1-(p-anisyl)methane (DIM-C-pPhOCH3), is the first identified Nur77 agonist[139]. DIM-C-pPhOCH3 activates GAL4-Nur77 chimeras expressing wild-type and the ligand binding domain of Nur77. In Panc-28 pancreatic cancer cells, Nur77 agonists decrease cell survival activating the cell death pathways, including tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and poly(ADP-ribose) polymerase (PARP) cleavage. Activation of TRAIL and PARP was further confirmed using Nur77 siRNA in Panc-28 cells. Nur77 agonists also inhibit tumor growth *in vivo* in athymic mice bearing Panc-28 cell xenografts[139]. DIM-C-pPhOCH3 arrests pancreatic cancer cell in G0-G1, by a Nur77-dependent, but KLF-4-independent, expression of cyclin-dependent kinase inhibitor p21. Interestingly, regulation of p21 does not require the presence of Nur77 response elements but involve a GC-rich promoter region and requires the presence of the Sp proteins Sp1

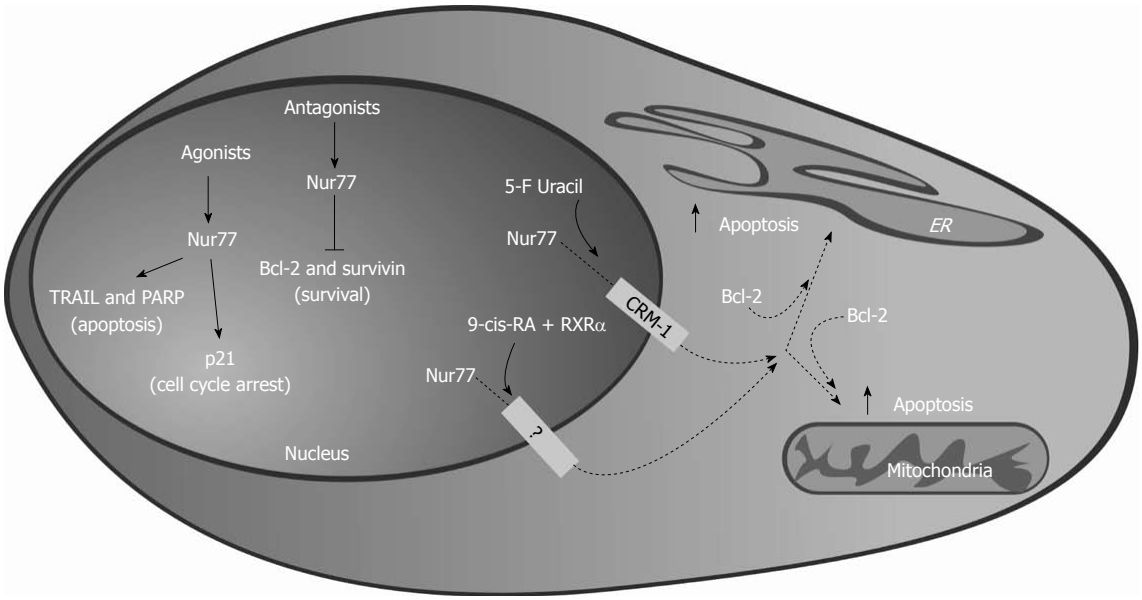

Figure 7 Nur77 acts as a tumor suppressor and as tumor promoting gene in pancreatic ductal carcinoma, inducing pro-apototic and anti-proliferative genes or repressing pro-survival genes. Dashed lines: Effects yet to be demonstrated in pancreatic ductal adenocarcinoma (PDAC). TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; PARP: Poly(ADP-ribose) polymerase; RXR: Retinoid X receptor; ER: Endoplasmic reticulum.

and Sp4, but not Sp3[140]. Microarray analysis of L3.6pL pancreatic cancer cells treated with DIM-C-pPhOCH3 demonstrated a NR4A1-dependent induction of genes associated with metabolism, homeostasis, signal transduction, transcription, stress, transport, immune responses, growth inhibition and apoptosis. Among the most highly induced growth inhibitory and proapoptotic genes were activating transcription factor 3 (ATF3), p21, cystathionase, dual specificity phosphatase 1 and growth differentiation factor 15. Furthermore, DIM-C-pPhOCH3 induced Fas ligand and TRAIL, the latter with a ATF3 dependent mechanism[141].

Although activation of Nur77 by specific c-DIM suggests that Nur77 is a tumor suppressor, experiments with the antagonist 1,1-*bis*(3'-indolyl)-1-(p-hydroxyphenyl)methane (DIM-C-pPhOH) suggest the contrary[142]. Blocking of endogenous Nur77 results in increased cell death and reduced cell proliferation; moreover expression of anti-apoptotic genes Bcl-2 and Survivin is also reduced. When administered *in vivo*, DIM-C-pPhOH inhibits tumor growth, acting on the same antiapoptotic markers observed *in vitro*. Survivin is overexpressed in pancreatic cancer[143,144] and its expression increases during PanIN progression to PDAC[144]. Transcriptional regulation of Survivin by Nur77 is Sp1-dependent, paralleling the p21 regulation[140], and it is co-regulated by p300. Thus, activation of nuclear Nur77 by the agonist DIM-C-pPhOCH3 or inactivation by the antagonist DIM-C-pPhOH reduces proliferation and induces apoptosis through two different transcription pathways: the first involves the induction of expression of apoptosis promoter genes such as p21 and TRAIL, whereas the latter is dependent on suppression of pro-survival genes. Consequently, Nur77 acts both as a tumor suppressor and as a tumor promoting gene in pancreatic cancer (Figure 7).

Interestingly, Nur77 may act as an apoptotic inducer agent in several cancer cells after nuclear export[96,97,145-147]; although not yet described in PDAC it is conceivable that this extra-nuclear action may also be present in pancreatic cancer cells. Inducers of apoptosis, including 5' -fluorouracil which is used in PDAC treatment, stimulate nuclear export of Nur77 mediated by the export receptor CRM1[145,147]. NR4A1 nuclear export may also be induced by 9-*cis*-RA, requires RXRα as a carrier[97], and targets Nur77 to mitochondria[96]. Despite lacking classical mitochondria targeting sequences, Nur77 might translocate to mitochondria in response to cell death stimuli, through interaction with anti-apoptotic Bcl-2[147]. Bcl-2 acts forming channels in the mitochondria membrane to regulate apoptosis[148]. The interaction Bcl-2/Nur77 is mediated by the N-terminal loop of Bcl-2 and by the NR4A1 LBD: this binding induces a conformational change that exposes the BH3 region of Bcl-2, resulting in its transformation in an inducer of apoptosis[147]. Interestingly, Nur77 may also translocate to other organelles, specifically endoplasmic reticulum (ER). Cell treatment with CD437 induces a nucleus-cytoplasmic translocation of Nur77, followed by ER localization: this requires again the interaction with Bcl-2 and triggers the release of Ca^{2+} from ER inducing apoptosis. Again, this effect has been demonstrated in human neuroblastoma, esophageal squamous carcinoma and hepatocarcinoma cells[145,146] but not in PDAC cells (Figure 7).

LRH-1 in pancreatic cancer

LHR-1 (NR5A2) is an orphan NR that is essential during development and necessary in the adult for the function of the pancreas, liver, intestine, and ovary[149,150]. LHR-1 recognizes specific DNA sequences to whom it binds as monomere[151].

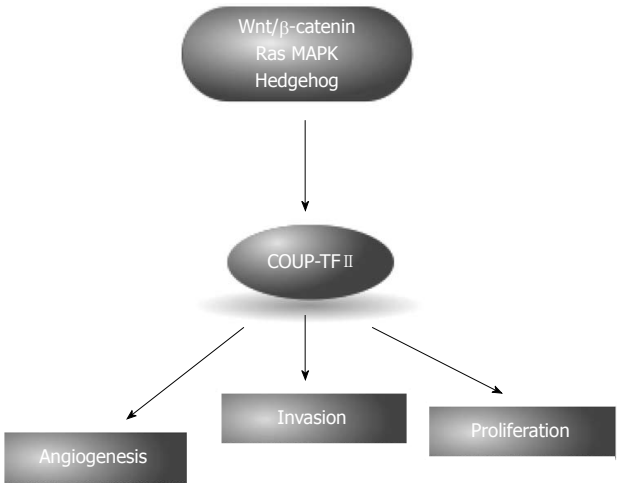

Figure 8 Chicken ovalbumin upstream promoter transcription factor Ⅱ is involved in the regulation of angiogenesis, invasion and tumor proliferation. Expression of chicken ovalbumin upstream promoter transcription factor Ⅱ (COUP-TFⅡ) is induced by several pathways altered in pancreatic ductal carcinoma, including Wnt/β-catenin, RAS-MAPK and Hedgehog.

The status of LHR-1 as "orphan" is debated and some scientists suggest that it may be classified as "adopted"[132,151]. The structure of the mouse LHR-1 LDB shows an active conformation with a large hydrophobic, but empty, ligand binding pocket resulting in a constitutive active receptor[132]; instead, the crystallographic analysis of the human LHR-1 revealed the presence of phosphatidyl inositol in the binding pocket[151]. Phosphatidyl inositol is required for activation[151] but it is not clear if it may enter and leave the pocket acting as a proper ligand[132]. Nonetheless, new molecules acting as antagonists have been recently identified by screening of commercially available compounds[152].

Chromatin immunoprecipitation-seq and RNA-seq analyses revealed that LRH-1 directly induces expression of genes encoding digestive enzymes and secretory and mitochondrial proteins and cooperates with the pancreas transcription factor 1-L complex in regulating exocrine pancreas-specific gene expression[153].

LHR-1 is important in maintaining acinar identity but is not required for acinar development[154] and mice with a selective deletion of LHR-1 in the pancreas did not display histological abnormalities[153-155]. A genome-wide association study conducted in pancreatic cancer patients and unaffected controls identified 5 SNP in the vicinity of NR5A2 associated with the risk of PDAC[156], that were confirmed by later studies[47,157,158].

In normal human pancreas, the LRH-1 protein is expressed at low levels in the nucleus and cytoplasm of both acini and ducts cells; in contrast PDAC show heightened levels of the protein and in some neoplastic cells the receptor appeared to localize predominantly in the cytoplasm[159]. An increased presence of LRH-1 was also detected in the acinar cells affected by pancreatitis and in PanIN lesions[159]. Overexpression of the receptor was also detected in pancreatic cancer cells *in vitro*[159].

Treatment of pancreatic cancer cells *in vitro* with LHR-1 antagonists or with LHR-1 siRNA significantly inhibits cell proliferation inducing a G0/G1 block associated with a reduction of of cyclins D1 and E1[152,159], suggesting that *in vitro* LHR-1 promotes tumor proliferation. *In vivo*, selective inactivation of one NR5A2 allele in pancreatic epithelial cells is sufficient to cause impaired recovery from pancreatitis[154] and conditional pancreatic deletion of LHR-1 leads to destabilization of the mature acinar differentiation state, increased inflammation, ADM and loss of regenerative capacity following acute caerulein pancreatitis[154,155]; loss of both alleles also dramatically accelerates the development of oncogenic Kras driven ADM and PanIN lesions[154,155].

The *in vivo* studies clearly show that LHR-1 inhibits the ductal transformation of adult acinar cells by mutant Kras and prevent PDAC progression; however *in vitro* studies show that NR5A2 promotes, instead of inhibiting, tumorigenesis. It has been hypothesized that NR5A2 exercises an inhibitory action in the early phase of PDAC development, blocking RAS with unknown mechanisms, while it will have an opposite effect later on[160].

COUP-TFⅡ expression predicts survival in PDAC

COUP-TFⅡ is a orphan nuclear receptor encoded by the NR2F2 gene localized in the chromosome region 15q26, a region frequently amplified in pancreatic cancer[14], and it is a down target of multiple pathways altered in pancreatic cancer[46,161-163]. In mouse two different transcription variants are described whereas in human at least four different variant are expressed. They differ in the N-terminal region and only one variant presents the structural features of NR being the others without the DBD. The role of these variants is not fully understood and two recent papers gave contradictory results describing one of the DBD lacking forms acting either as enhancer of COUP-TFⅡ transcriptional activity or as a repressor, increasing the cytoplasmic localization of full length COUP-TFⅡ, suggesting a cell specific function for this truncated NR[164,165]. COUP-TFⅡ exists in a autorepressed conformation that prevents recruitment of coactivators, and might respond to retinoids that promote COUP-TFⅡ to recruit coactivators[166]. Full length COUP-TFⅡ exerts an important role during development and in adulthood[167], and it is implicated in the progression of various type of cancers[168]. COUP-TFⅡ is expressed at low levels in adult normal exocrine pancreas[168,169] and recently we demonstrated its involvement in pancreatic cancer *in vitro* and *in vivo*[168] (Figure 8). COUP-TFⅡ was expressed in 69% of tested primary samples correlating with the presence of lymph and distant metastasis as well as clinical stage; PDAC patients stained positive for the NR showed a significant reduction of survival compared to NR-negative patients. *In vitro* silencing of COUP-TFⅡ reduces the cell growth and invasiveness and it strongly inhibits angiogenesis, an effect mediated by the regulation of VEGF-C. The reduced proliferation is associated with a block in G1 and

Table 2 Expression of nuclear receptors and clinical trials

Nuclear receptor	Expression in primary PDAC	Clinical trials and results
PPAR (-α, -β, -γ)	PPARα: Unknown PPARβ/δ: overexpressed; expression correlates with tumor stage, recurrence, and distant metastasis PPARg: maybe overexpressed; expression correlates with shorter overall survival	None for PPARa and β/δ; PPARγ: TZD apparently reduce the risk of PDAC but PPARγ agonists do not improve survival
RAR and RXR (-α, -β, -γ)	Yes (with the exception of RARb that is apparently lost during cancer development)	13-*cis*-RA in combination with IFN-γ: prolonged stable disease as well no improvement have been reported
AR	Yes	Phase Ⅱ trials with the antagonist flutamide: increase in survival as well no effect have been reported
ER (-α, -β)	Yes	Tamoxifen with no benefit
NR4A1, NR4A2, NR4A3	NR4A1: overexpressed NR4A2 and NR4A3: unknown	None
LHR-1	Overexpressed	None
COUP-TFⅡ	Overexpressed; expression correlates with shorter overall survival, tumor stage, and presence of metastasis	None

PPAR: Peroxisome proliferator-activated receptor; PDAC: Pancreatic ductal carcinoma; TZD: Thiazolidinediones; IFN: Interferon; RAR: Retinoic acid receptor; RXR: Retinoid X receptor; AR: Androgen receptor; ER: Estrogen receptor; LHR-1: Liver receptor homologue-1 receptor; COUP-TFⅡ: Chicken ovalbumin upstream promoter transcription factor Ⅱ.

decreased expression of E2F1, but not apoptosis; moreover, COUP-TFⅡ silencing reduces OCT4 and increases Nanog expression. *In vitro* effects were confirmed in nude mice where COUP-TFⅡ silencing reduces tumor growth by 40%[168].

CONCLUSION

PDAC is a devastating disease originating from well defined genetic alterations. However, due to the its subtle nature, the lack of efficient diagnostic methods and of effective drugs it is a deadly disease with a dismal prognosis. NR are ligand-regulated transcription factors functionally involved in important cellular functions ranging from regulation of metabolism, to growth and development. Given the nature of their ligands, NR are very tempting drug targets and their pharmacological modulation has been widely exploited. There are now clear evidences that both classical ligand-activated and orphan NR are involved in the pathogenesis of pancreatic cancer disease from its very early stages. From the review of the literature PPARs, RARs, RXRs, AR, ERα and ERβ and the orphan NR Nur, COUP-TFⅡ and LHR-1 show striking connections with PDAC development, that for certainty will need more experimental confirmations, especially for the orphans. Although clinical application of NR modulators in the PDAC treatment still suffers from failure (Table 2), a more comprehensive analysis of NR action in PDAC could lead to the identification of novel therapies for this disease.

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