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REVIEW ARTICLE

Urotensin-II Receptor: A Double Identity Receptor Involved in Vasoconstriction and in the Development of Digestive Tract Cancers and other Tumors

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Abstract: Urotensin II and Urotensin-II receptors are important molecular factors that regulate vasoconstriction and all the diseases that are linked to abnormalities in blood pressure regulation (*i.e.*: hypertension, kidney diseases, cirrhosis *etc.*). Recently, Urotensin II and its receptor have also been involved in metabolic syndrome, diabetes and schizophrenia. Recent strong findings suggest that Urotensin II and its receptor are involved in the onset and development of different epithelial cancers. Indeed, it was reported that cell growth, motility and invasion in human breast, bladder, prostate, colorectal and glioblastoma cancer cells were regulated by Urotensin II and Urotensin-II receptor axis**.** This axis also regulated focal adhesion kinase and small Guanosine-5'-triphosphate binding proteins that likely had a role in motility and invasion mediated by Urotensin-II receptor. Additionally, its expression on tumour tissues is variably associated to the prediction of the clinical outcome of the patients and it can be considered an alternative molecular marker to be used as prognostic factor in human cancers. In conclusion, a new weapon in the treatment of human cancers is highlighting a new scenario for the future.

Keywords: Urotensin-II receptors, digestive tract cancers, cancer prognostic marker.

INTRODUCTION

Urotensin-II (U-II) has vasoactive properties, as well as Endothelin-1 (ET-1) and Adrenomedullin (AM), which possesses a significant vasoconstrictor activity, more remarkable than ET-1 in terms of potency [1]. U-II acts as a potent vasoconstrictor in humans [1, 2], although it has been originally isolated from urophisis of teleostean fish Gillichthys mirabilis [3, 4]. U-II has a somatostatin-like structure and has been mainly studied for its vasoactive physiological role, turning out to be an important molecule for the understanding and treatment of mechanisms that support diseases such as hypertension [1, 3, 5, 6]. The biosynthesis of this peptide-hormone starts from a pre-pro-protein subjected to several post-translational processes mediated by proteasesnot yet well identified. Precursors of the active isoforms (pro-UT-1 and pro-UT-2) have not yet been correlated to specific biological effects and it is not clear whether these precursors could generate other molecules of biological interest.

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Human isoform of U-II is an undecapeptide and its C-term region is arranged in a cyclic esapeptide sequence due to a disulfide bridge between two cysteine residues [2]. To date, diverse U-II isoforms have been isolated from fish to amphibious, revealing, by structural analysis investigation, the high conservation of C-term region that is a site of prime importance for the function of the peptide [7]. Recently, a new peptide has been isolated from the rat, which is structurally akin to U-II due to equal cyclic C-term portion, A[CFWKYC]V, and for this reason named Urotensin-II related peptide (URP). This peptide has shown high affinity for human U-II receptor, as well as a very similar biological activity [8]. U-II interacts with a specific receptor that was investigated for its remarkable scentific interest. This receptor has been first identified as G protein coupled-receptor (GPR)-14 [9-11], an orphan receptor that belongs to GPCRs family [12]. Originally discovered in 1999 by Ames *et al.* in the rat, Urotensin-II receptor, also known as UT receptor (UTR), has exhibited structural analogies with somatostatin, opiate and galantine receptors [13]. Grieco and co-workers have studied the way somatostatin (SST)-14 and corticostatin-14 are able to activate UTR albeit in elevated concentration, but they have proved to be irrelevant towards physiological effects [2]. Moreover, this study has confirmed the role of U-II as the unique endogenous ligand that binds

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UTR with high affinity [2]. In humans, the minimum active sequence of U-II (hU-II) is represented by the fragment 4-11, H-Asp-[Cys-Phe-Trp-Lys- Tyr-Cys]-Val-OH, hU -II(4-11), characterized by potency and efficacy comparable to native whole sequence. U-II and UTR are expressed everywhere in human body. The function of these peptides is yet object of study for many tissue types; in other tissue types this function is only hypothesized. Particularly, elevated expression of U-II and UTR mRNA has been observed in cardiac tissue (atrial and ventricular myocytes, fibroblasts), arteries (mainly aorta), endothelial cells and smooth muscle cells (especially in vessels), liver, kidney and endocrine tissue [11, 14-16]. By immunoreactivity test, U-II has also been found in both central nervous tissue [17-23] and spinal cord motor neurons, intervening in neuromuscolar trasmission [24]. In fact, in the brainstem and spinal cord, cholinergic neurons have been found to express both UII and URP genes [25]. U-II and URP work through the interaction with a G-coupled protein receptor without significant differences in the affinity and signal transduction pathways activated. In the details, the connection of U-II with its receptor leads to the activation of Gq protein that activates protein kinase C (PKC), a serine threonine kinase, calmodulin and phospholipase C (PLC). PLC induces the formation of inositol trisphosphate (IP3) and diacylglycerol (DAG) that once activated, determines calcium release from sarcoplasmic reticulum, with consequent increase of intracellular calcium concentration. This increase is responsible for the vast majority of the effects linked to UTR activation including PKC stimulation [26, 27]. The interaction U-II/UTR would have vasoconstrictor effects mediated by activation of myosin light chain kinase 3 (MLCK), extracellular signal regulated kinase (ERK), a kinase regulating cell proliferation and survival, RhoA/Rho kinase (ROCK), as well as by PKC mediated-pathway that was previously described [28, 29]. Finally, Protein kinase B/Glycogen synthase kinase 3 beta (Akt/GSK-3beta) pathways and beta-catenin stabilization play an important role in the UII-mediated hypertrophy [30, 31]. Apart from its vascular effects, U-II causes contraction of smooth muscle cells of human small respiratory tracts and cat and mouse bronchial tubes [20, 32]. Transductional pathways and functional role of UTR are represented in Figs. (**1** and **2**), respectively.

UTR AND DISEASES

The possible role that U-II and UTR could have in promoting a remarkable number of diseases, also systemic, is becoming an interesting matter in scientific community.

Pulmonary arterial hypertension (PAH) is a fatal disease for which any progress in the treatment is decisive. It has been recently reported a study that compares the standard therapy based on ET-1 inhibitor bosentan with different dosages of U -II inhibitor palosuran [33] and a statistically significant difference for mean pulmonary arterial pressure (mPAP), U-II, ET1 levels, and pulmonary vascular pathology has been observed.

The authors concluded that U-II inhibitor and standard therapy showed similar efficiency [34]. Thirty-three pregnant women with hypertensive disorders and twenty-two healthy controls have been recently enrolled.

The authors found positive correlation between U-II and endoplasmic reticulum stress (ERS) markers expression level in placental tissues that also correlated with systolic blood pressure and proteinuria levels [35]. It was also reported a role for UTR in the determination of hypertrophyc cardiomyopathy in a rat model. In fact, the results of this study suggested a key role for U-II and the cyclic adenosine monophosphate-protein kinase A (cAMP-PKA) pathway in pressure overload-induced myocardial fibrosis [36]. On these bases, a highly potent UTR antagonist showed anti-hypertrophic effects both in rat models of infarction and in mouse model of pressure overload hypertrophy [37]. On the other hand, it was previously reported the involvement of β-arrestin in UTR-induced Epidermal Growth Factor Receptor (EGFR) trans-activation [38] that protected against pressure overload-induced hypertrophy [39]. Based on its function on endothelial cells, UTR expression was also studied in relation to human corpus cavernosous (HuCC) function. In fact, UTR was found in human and rat corpus cavernosum. In HuCC UTR was expressed on endothelial cells. U-II induced a significant endothelium- and -NO-dependent relaxation of HuCC strips. Moreover, pressure in anesthetized rats corpus cavernosum increased significantly [40]. On these bases, two agonists of U-II were injected in the Rat Corpus Cavernosous (RCC) and the intracavernous pressure (ICP) was measured.

Intracavernously injected U-II (0.03-1 nmol) and the agonists P5U (0.03-1 nmol) or UPG84 (0.03-1 nmol), increased ICP. Particularly, ICP was significantly modulated by P5U compared to U-II [41]. The elevated expression of U-II in kidney has led to make a hypothesis of its pathophysiological role in glomerular filtration [42]. It was recently discovered that UII-induced store-operated Ca(2+) entry (SOCE) causes contraction in murine glomerular mesangial cells (GMC) and that UTR [43] activation recruits regulator of G-protein signaling (RGS2) to GMC membrane that negatively regulates UTR transduction pathway [25]. On the other hand, genetic polymorphisms of U-II/UTR axis have not been found to be associated to blood pressure control regulated by renal function [44]. Moreover, U-II and UTR are more highly expressed in tubules than in GMCs suggesting its role in the filtration mechanisms. Because of the abundant U-II expression in sclerotic areas, U-II may be related with glomerular sclerosis for its inflammatory properties or by acting as a growth factor [45].

These experimental observations lead to consider U-II as hypertensive disease multi-systemic mediator. Indeed, as reported, it would act at multiple levels: through the induction of vasoconstriction, proliferation of vascular smooth muscle cells by inducing myocardial hypertrophy; additionally it is involved in regulation mechanisms of glomerular filtration and glomerulosclerosis. In this last case, U-II could be considered as a factor that links hypertension to the development, over time, of glomerular sclerosis, by producing a vicious circle that promotes hypertension itself.

Furthermore, there is an important correlation between liver disease and U-II expression levels. Indeed, in cirrhotic patients U-II mRNA expression and levels of protein in plasma increase in relation to the severity of disease and portal pressure levels [46, 47]. Consequently, serum U-II may be used as surrogate marker of portal hypertension and, in

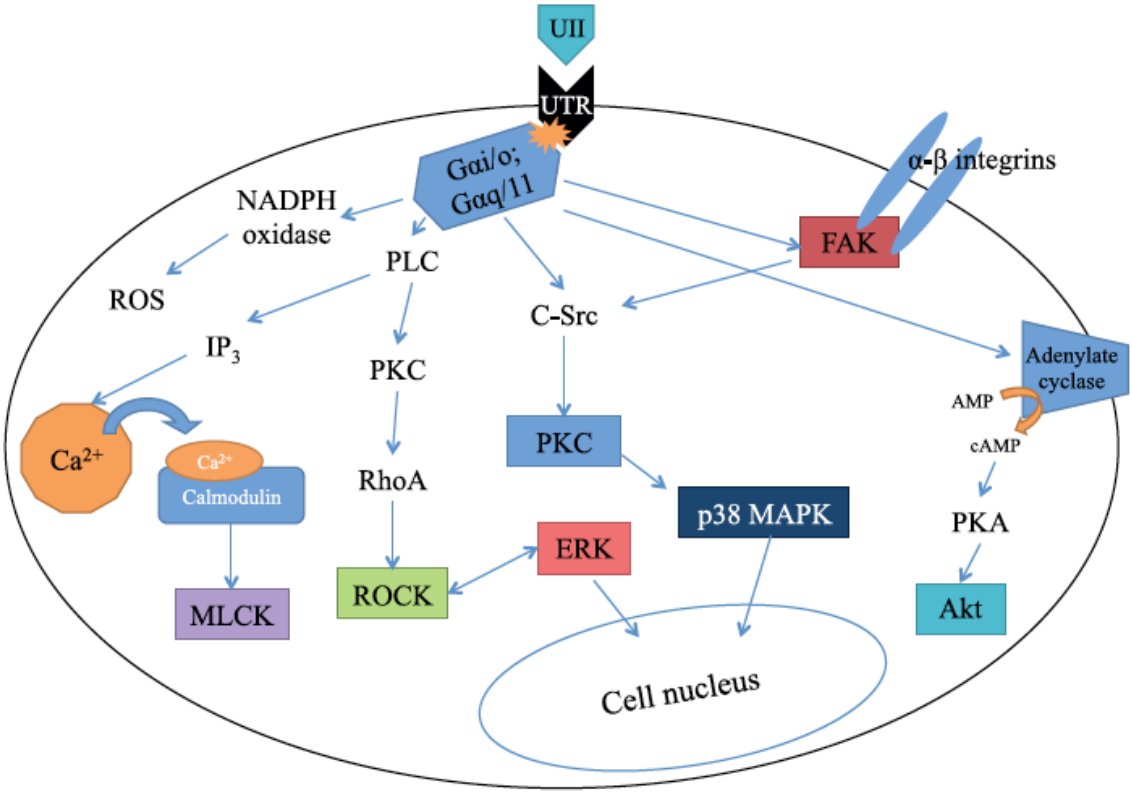

Fig. (1). Transductional pathways of Urotensin-II Receptor. PKC: protein kinase C; ERK: extracellular signal-regulated kinase; ROCK: RhoA/Rho kinase; PLC: phospholipase C; IP3: inositol-1,4,5-trisphosphate; MLCK: myosin light-chain kinase; NADPH: nicotinamide adenosine dinucleotide phosphate; ROS: reactive oxygen species; c-Src: Src kinase; UTR: UII receptor; UII: Urotensin-II; Gq/i, subtype of G protein; MAPK: mitogen-activated protein kinase; FAK: focal adhesion kinase; cAMP: cyclic adenosine monophosphate; AMP: adenosine monophosphate; PKA: protein kinase A; Akt: Protein kinase B.

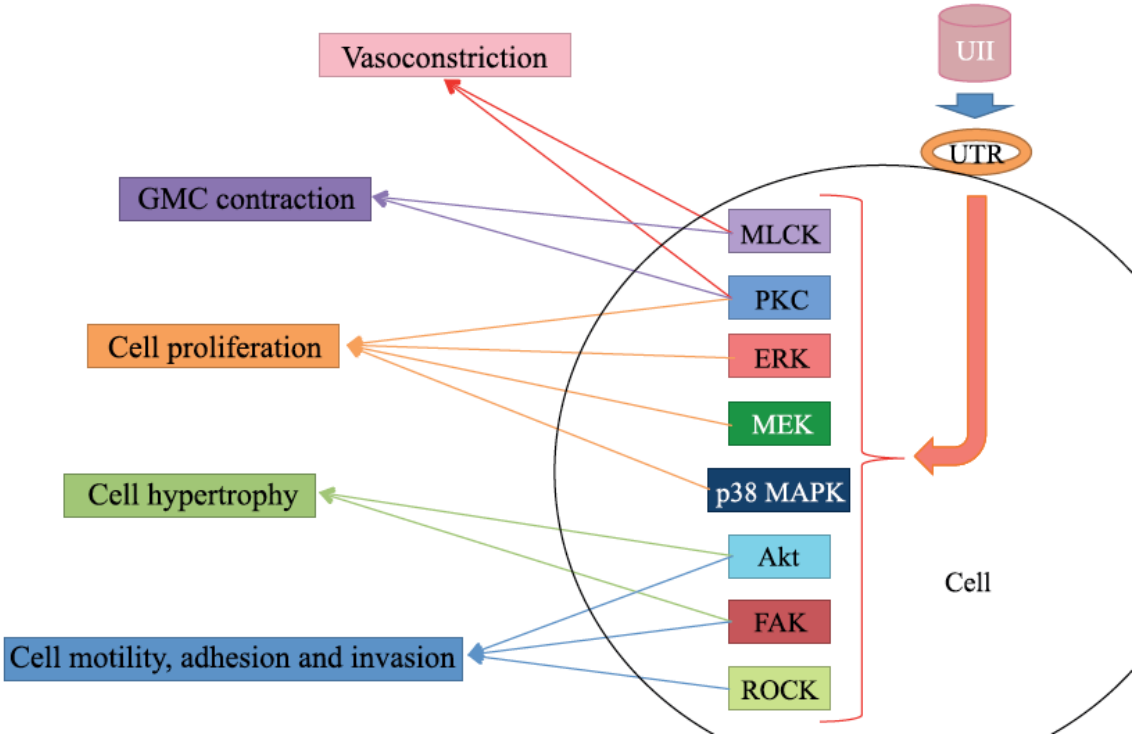

Fig. (2). Correlation between kinases activated by Urotensin-II Recptor and their biological functions. UII: Urotensin-II; UTR: Urotensin-II Receptor; MLCK: myosin light-chain kinase; PKC: protein kinase C; ERK: extracellular signal-regulated kinase; MEK: Mitogen-activated protein kinase; MAPK: mitogen-activated protein kinase; Akt: Protein kinase B; FAK: focal adhesion kinase; ROCK: RhoA/Rho kinase.

addition to parameters used for Child Pugh evaluation and Model for End-Stage Liver Disease (MELD) score, it could be useful to identify better patients with a severe hepatopathy. In this way, a reconsideration of the current prognostic evaluation, applied to patients with liver diseases, may be possible.

Nowadays, the etiology of liver disease is radically changing and we are witnessing a constant increase of patients affected by metabolic syndrome and liver steatosis [48]. Recently, a possible role of U-II in determining metabolic syndrome [49] has emerged. In patients with type 2 diabetes mellitus an elevated level of [50] U-II has been observed both in plasma and urina [13, 51, 52]. On these bases, obese ob/ob mice treated with the UTR antagonist SB657510 showed significant improvements in glucose levels, blood pressure, hyperlipidemia, cardiac function, intracellular Na(+) and Ca(2+) and a decrease in weight and sodium/hydrogen exchanger 1 (NHE- 1) protein expression compared with vehicle [53] ($P<0.05$). These data demonstrate the possible translation of experimental results in the pharmacological treatment of metabolic syndrome [54], since they demonstrate that switching off the signal activated by U-II, through a therapy with antagonist for UTR, leads to an improvement of important indices of metabolic homeostasis, as well as to be able to take position against an important component of metabolic syndrome: arterial hypertension. In the last years, the same authors have demonstrated that in mice knockout for UII gene (UIIKO) body mass, visceral fat, blood pressure and an increase of insulin and glucose tolerance significantly decreased compared to wild-type mice [55, 56].

For a summary of the evidence of UTR involvement in non neoplastic diseases (Table **1**).

In recent years, another important function of U-II and its receptor has been investigated: the ability to modulate cell proliferation, invasion, motility and metastatization of tumour cells. In fact, many researchers have attempted to figure out the mechanisms by which these peptides could promote cell proliferation in tumour diseases. For instance, an important role is carried out in adrenocortical tissue in determining primary aldosteronism; in lymphangioleiomyomatosis (LAM) cells in patients with LAM; colon and prostate adenocarcinoma, bladder carcinoma; in rat models with diethylnitrosamine-induced hepatic precancerous lesions [57] and human hepatocarcinoma (HCC), as well as breast carcinoma [58-65].

ROLE OF U-II→UTR AXIS IN THE REGULATION OF CANCER PROLIFERATION AND DEVELOPMENT

The role of peptides with vasoactive properties, including ET-1 and U-II, has been [66] investigated for a long time. About these peptides, it has been recently studied the possibility to induce cell proliferation by pathways associated with their receptors. In 2000 some groups of researchers confirmed the expression of these molecules for a few human cancer types [67, 68]. In a study by Takahashi *et al.* [69], it has been studied the expression of these three proteins and their related receptors in eight tumoral cell lines: glioblastoma (T98G), neuroblastoma (IMR-32, NB69), choriocarcinoma (BeWo), adrenocortical (SW-13), colon (DLD-1), cervical carcinoma (HeLa, VMRC-RCW).

The analysis with RT-PCR has demonstrated mRNA expression of ET-1 in seven cell lines, with the exception of BeWo. mRNA of endothelial "type a" receptor endothelin (ETa) is expressed in NB69, IMR-32, T98G cell lines and weakly in the resting cells. "Type b" receptor endothelin (ETb) is found in IMR-32, NB69, BeWo cells and slightly in T98G and HeLa cells.

Immunoreactivity for ET-1 has been observed in six out of eight cell lines. Cells have also been treated with antagonists of endothelin receptor: BQ-610, antagonist of ETa and BQ788, blocking ETb receptor. This has allowed identifying which of the two receptors is mainly involved in the mecha-

Table 1. Urotensin-II Receptor involvement in non neoplastic diseases.

Disease	Involvement Evidence	Drug	Ref
Pulmonary arterial hypertension	Evaluation trial of U-II inhibitors *vs.* ET-1 inhibitor bosentan	Palosuran	[34]
Pulmonary arterial hypertension	Positive correlation between U-II and endoplasmic reticulum stress markers expression level in placental tissues	ND	[35]
Hypertrophyc cardiomyopathy	Myocardial fibrosis in chronic pressure-overload rats	Palosuran	[36-39]
Erectile dysfunction	Modulation of rat Intra-Corpus Cavernosus pressure	P5U and UPG84	[41]
Kidney disease	Role in the progression of glomerular sclerosis	ND	[45]
Cirrhosis	U-II mRNA expression and levels of protein in plasma increase in relation to the severity of disease and portal pressure levels	ND	[46, 47]
Metabolic syndrome	obese ob/ob mice treated with the UII receptor antagonist showed ameliorated syndrome parameters UII gene knockout in mice (UIIKO) induced a significant decrease ofmetabolic syndrome severity	SB657510	[55]

U-II: Urotensin-II; ET-1: Endothelin-1; ND: not detected.

nism of activation of cell proliferation. This research has led to three important results: i) ET-1 is produced by different tumoral types and can act as autocrine and paracrine growth stimulant for tumoral cells through a signal mainly mediated by ETa receptor activation; ii) AM is produced and secreted by various endocrine and non-endocrine cancers and it has a stimulating effect on tumoral cell growth; iii) U-II required additional studies with the aim to clarify its role in proliferative pathologies. Over time new proofs have been acquired about its role in promoting proliferative diseases carried out by U-II/UTR, which have led to consider these molecules as important pharmachological targets for future antitumoral therapies [67-69].

PROSTATE CANCER

Adenocarcinoma is the most frequent primary cancer in the prostate with an incidence very variable in relation to different epidemiological data analyzed; however, these studies confirm an increase of prevalence in relation to the advance of the age. The severity of prostate carcinoma is extremely variable, since there are conditions completely benign and other with a very severe prognosis [70-72]. Nowadays, the main prognostic index is Gleason's score, which conveys the experience of pathologist [73]. Recently, Grieco *et al.* have investigated the potential role of U-II and UTR in prostate adenocarcinoma [60]. Particularly, the objectives of the study were: i) to evaluate *in vivo* UTR expression on prostate tissue samples and *in vitro* on the three classic cell lines for the study of prostate carcinoma (androgen- independent DU145, PC3; androgen-dependent LNCaP); ii) to study *in vitro* the effects of U -II and Urantide on the cell growth, migration and invasion of DU145, PC3 and LNCaP cells. UTR expression evaluated with Western Blot was higher in LNCaP cells, and lower in androgen-independent DU145 and PC3 cells. UTR mRNA expression evaluated by RT- PCR was correspondingly higher in LNCaP cells than in the other two lines. UTR expression has also been evaluated with immunohistochemistry on 195 prostate tissue samples deriving from biopsy or prostatectomy for adenocarcinoma. This protein was moderately expressed in benign hypertrophic prostate tissues, whereas in cancer its expression showed a variation on the basis of disease grading. Specifically, UTR was expressed with elevated intensity in well differentiated prostate adenocarcinoma, Gleason score <7, whereas it was less expressed or even absent in adenocarcinomas with a more advanced grading, Gleason >7. It was concluded that, like Gleason score, also the evaluation of tissue expression of UTR represents an important prognostic index; particularly the lack of expression, evaluated by survival statistical models, confirmed a worse long term prognosis [60]. To investigate the role of UTR in cell motility and invasion, LNCaP cell line was treated with Urantide.

Both quantitative and qualitative analysis for the measure of these parameters have been performed by scanning electron microscope (SEM) and computer assisted optical microscopy. After treatment with Urantide, the analysis of the outcomes has demonstrated a dose-depedent decrease of the area occupied by migrating and invading cells. Urantide also has effects on adhesion factors and many of these regulate processes of extracellular matrix/cytoskeleton interaction responsible for cell-proliferation, migration and invasion. Focal adhesion kinase (FAK) is a non receptorial tyrosine kinase protein, which promotes both cell motility and invasion. Activation of FAK increased in LNCaP cells after induction of migration. The treatment with Urantide for 24 h reduced in a significant fashion pFAK expression in these cells (30-50%), as well as cluster of differentiation (CD)61 and CD11 expression (40%) (integrin $\alpha v\beta 3$, integrin $\beta 2$), both involved in processes of cell adhesion. Similar outcomes were obtained with UTR gene silencing obtained through cell transfection with a short hairpin RNA (shRNA). However, the association of Urantide with shRNA did not cause effects higher than the treatment with either Urantide or shRNA alone. Finally, the analysis of survival statistical models confirmed that patients with a higher UTR expression level (Gleason score <7) have a life expectancy better than those with lower UTR expression (Gleason –>7) [60]. These data were confirmed by another study in which 58 subjects with radical prostatectomy were enrolled. Multivariate analysis suggested that Gleason upgrading and pathology upstaging correlated with low UTR expression (OR: 10.3, 95% CI: 1.55-68.4 and OR: 11.1; 95% CI: 1.23-100.48, respectively) [74]. UTR expression level, evaluated by using immunohistochemistry, represents a prognostic marker independent of Gleason score. This is useful to differentiate better advanced cancer types, since this parameter is independent of pathologist experience.

Moreover, the explanation of lower UTR expression in advanced stages may be due to the fact that undifferentiated carcinoma could benefit from signals different from that mediated by UTR for the activation of proliferative and metastatic mechanisms,either through intracellular Ca^{+2} increase or constitutive FAK activation and mitogen-activated protein kinase (MAPK). This is related to a higher cell atypia and different gene expression, also confermed by simultaneous loss of androgenic dependence.

BLADDER CANCER

Transitional cell carcinoma of bladder is classified in two main types, based upon both histology and prognosis: non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC), and it is the fourth among the most frequent malignant neoplastic diseases worldwide [75]. Currently, histopathological stage and grading are the most important prognostic factors for bladder cancer [76]. Despite these two parameters are used for a long time, they depend on the experience of the pathologist who analyzes samples [77]. Therefore, alternative prognostic markers are strongly warranted. In a recent study, Franco *et al.* [62] have investigated the role of U-II/UTR-related pathways on the ability to modulate proliferation, migration and invasion of different transitional cancer cell lines. Moreover, the authors have evaluated *in vivo* the expression levels of UTR in order to attribute a prognostic value, predictive of relapse and able to differentiate NMIBC from MIBC. The evaluation of UTR expression levels has been carried out *in vivo* on samples from 159 patients. UTR expression was higher in NMIBC than MIBC; moreover, MIBC patients showed a higher number of negative samples for UTR expression than NMIBC.

Relapses have been observed in 75 patients (47%). Recurrent cancer grading has been the same of primary cancers

in 71 patients, whereas 4 patients have demonstrated a grading progression. Elevated UTR expression was related to cancer grading, being higher in low grade than in high grade cancers. Moreover, UTR expression was lower in reccurent cancer, also highlithing a prognostic role for UTR.It has also been recorded a significant association between low UTR expression and the short time of recurrence. This observation may lead to a whole revision of prognostic indices, evaluated at the moment of diagnosis of this type of neoplasia, through biopsy. An early diagnosis in patients with a higher risk of disease relapse could lead to a new planning of the current method of post-surgery follow-up of tumour ablation with significant advantages in terms of lives and health economics.

Thereafter, the biological effects of U-II agonist/ antagonist peptides (U-II, UPG84/UPG83, UPG85, Urantide) were evaluated *in vitro*. The treatment of MCR, RT112, T24 and HT1376 bladder cancer cell lines with U-II for 72 h did not induce growth stimulation. On the other hand, the superagonist UPG84 determined growth inhibition in all assessed cell lines, with the exception of RT112. The authors have hypothesized that this effect was likely due to receptor internalization and consequent UTR post-transductional down-regulation. Indeed, UTR once activated, also promotes beta-arrestin translocation to the cell membrane. Beta-arrestin is involved in the internalization of the receptor and prevents the coupling of G-protein from its receptor, both blocking G protein-mediated (Gq, Gi, Go, G13) pathways and leading to a fast down-regulation of UTR effector system [13]. Urantide determined cell growth inhibition in RT112 and T24 cells, whereas it did not induce significant effects in both HT1376 and MCR cells [62]. To evaluate effects of UTR on both motility and invasion, T24 e RT112 cells were incubated for 48 h with Urantide, that induced an about 35% and 50% reduction of migratory and invasive capacity, resepectively. Moreover, cell transfection of shRNA for UTR caused an inhibiting effect of either 45% or 56% on motility and invasion, respectively. However, the treatment of shRNA-UTR-transfected cells with Urantide did not determine additional inhibition. This last effect could be explained by UTR downregulation from cell surface, confirming that the addition of Urantide is able to produce an inhibitory biological effect,but only through interaction with its receptor and not with other surface structures [62].

DIGESTIVE SYSTEM CANCER

Hepatocellular Carcinoma

The occurrence of HCC is generally due to a chronic disease that, through chronic tissue inflammation, slowly leads to parenchymal damage, progressing in sequential gene hits, which induce cancer formation and development. In fact, after 15-20 years, the continuous parenchymal damage leads to cirrhosis and HCC development.

U-II and UTR are found in all human tissues, such as liver [14, 78-80] which would be also responsible for U-II plasma levels [81]. In fact, high U-II levels in plasma were found in chronic hepatic disease [82]. In a study by Wang *et al.*, performed on mice [63], an association between precancerous lesions induced by the exposure to diethylnitrosamine (DEN) and the level of plasmatic and tissue UTR and U-II expression was investigated. The aim of the report was the identification of the intracellular signaling pathways of UTR that could mediate cell proliferation, as well as to evaluate if exposure of mouse oval cells to U-II *in vitro* was able to stimulate their growth. The authors induced precancerous lesions in animal liver by DEN introduction in abdominal cavity. At the end of the treatment, mice were sacrificed, liver was removed and blood was collected; these samples were used in order to confirm the presence of precancerous lesions. Higher U-II and UTR liver tissue expression was recorded in diseased mice if compared to normal animal group and similar results were also found for blood circulating U-II levels. Analysis with real time Polymerase Chain Reaction (RT-PCR) showed anabout 101.2% increase of UTR mRNA compared to controls. The authors evaluated, *in vitro*, if the incubation of HOCs cells with U-II was able to induce cell proliferation. HOCs cells represent a useful model for the study of HCC *in vitro*. After 24 h incubation cell growth stimulation was observed. Finally, in order to assess the trasductional pathways involved, cells were incubated at different times with U-II together with PKC (Calphostin C) or mitogen-activated protein kinase kinase (MEK) (PD98059) or MAPK (SB203580) inhibitors. The results suggested that the effects on cell growth inhibition were PKC and MEK- dependent. Yu *et al.* [64] have recently reported both higher mRNA and protein expression of U-II and UTR in cancer tissue than in healthy tissues of the same patients. These data were paralleled by higher levels of PKC, extracellular-signal-regulated kinase (ERK) and p38 MAP kinase phosphorylation in cancer samples if compared to healthy ones. These results were reproduced also *in vitro* in HCC BEL-7402 cells that expressed high levels of UTR. The incubation of BEL-7402 cells with U-II increased phosphorylated PKC, ERK, and p38 MAPK and stimulated cell growth. Finally, BEL-7402 cells were incubated with U-II with or without PKC, ERK, p38 MAPK inhibitors of GF109203x, PD184352, SB203580 respectively, in order to confirm if this phosphorylation was required to induce cell proliferation. The growth-promoting effect induced by U-II was partially abolished by the use of GF109203x, PD184352, SB203580, confirming that the pathways mediated by PKC, ERK, and p38 MAPK are, at least in part, involved in U-II-induced cell proliferation. The high level of serum U-II that can be observed in chronic liver diseases, may lead to hypothesize that liver pathology, especially cirrhosis, could also act through a mediation carried out by U-II in determining neoplastic pathology. For this reason, the intervention with therapies in the break of this ring of connection,could outline the prevalence of HCC in patients with liver diseases again.

Colorectal Carcinoma

Colon cancer is the second deadly tumour in men after lung cancer and third in women after breast and lung cancer [83]. Colon adenocarcinoma causes death in approximately 25 subjects out of 100,000/year [84, 85], and it represents a great epidemiological and economic problem for health and social expenses; therefore, the study of new preventive and therapeutic strategies is strongly warranted. Patient survival and therapeutic strategies depend on the disease stage at the moment of diagnosis.

Federico *et al.* [59] have demonstrated, by using Western Blotting, that UTR is expressed in all four evaluated colorectal cancer cell lines (LOVO, HT-29, SW620, COLO and WIDR) with higher expression in WIDR cells. Tissue samples of 114 patients were analyzed with immunohistochemistry, respectively 52 men and 62 women divided into 48 patients with adenocarcinoma, 21 with adenoma and 45 with normal colon tissue. UTR expression was low in normal colon tissue (5-30%), medium in adenomatous polyps (30-48%), and high in adenocarcinomas (65-90%). Moreover, in well differentiated (*i.e.* G1) cancer, 85% of cells was UTR positive compared with poorly differentiated (*i.e.* G3) cancers that showed 70% of UTR positive. UTR mRNA expression was 3-fold higher in patients with adenomatous polyps than in control tissues, and up to 8-fold in patients with adenocarcinoma. Normal colon tissue fragments extracted in patients with adenocarcinoma showed UTR expression levels comparable to the expression of patients without cancer. The biological effects of U-II and its antagonistic or agonistic peptides were also investigated on colorectal cancer cell lines. U-II increased cell growth in HT29, LOVO and WIDR lines by 20%, 30% and 60% respectively, whereas urantide induced an about 35%, 20% and 40% cell growth inhibition in LOVO, HT29 and WIDR lines, respectively. UPG83 and UPG85 also inhibited cell proliferation of HT29 cells by 30% and 35%, of LOVO cells by 45% and 50%, of WIDR cells by 45% and 55%, respectively. High U-II concentrations of 100 nM for 72 h caused an about 60% growth inhibition likely due to receptor down-regulation that can make cells insensitive to proliferative effects induced by the activation of UTR pathway. U-II, regulating Ca+2 intracellular levels, can be responsible for changes of tumour cell cytoskeleton that is required for metastatic process. On these bases, the authors evaluated the effects derived from UTR block with either urantide or shRNA on migration and invasion of colon cancer cell lines (LOVO and WIDR). shRNA led to an efficient reduction of UTR expression on cell surface after 24 h from transfection. Transfected LOVO cells showed a reduction of 35% and 45% migration and invasion, respectively, as compared with non-transfected cells. Similarly, in WIDR cells, transfection caused an about 70% and 80% decrease of motility and invasion.After 48h, urantide (100 nM) induced an about 50% decrease of both migratory and invasive properties in WIDR cells and LOVO cells, whereas treatment of anti-UTR shRNA-transfected LOVO and WIDR cells with 100 nM urantide did not cause any advantage in terms of motility and invasion inhibition. From these observations, it is possible to deduce that, in the initial steps of cancerogenesis process, the interaction U-II/UTR carries out an important role in the progression of neoplastic disease. Tumour tissue could produce a higher amount of U-II, and at the same time, it could become more susceptible to U-II. This is related to a higher expression of both mRNA for UTR and UTR itself. Therefore U-II may be considered as autocrine and paracrine factor of tumour expression.

Breast Cancer

Breast cancer is the most frequent and deadliest cancer in U.S. women, resulting in an estimated 40,730 new deaths in 2015 [86]. The stage of disease at the diagnosis influences long-term survival of breast tumor patients: the 5-year survival rate is 99% for localized disease, 85% for regional stage, and 25% for distant-stage tumor [87]. Therefore, attempts to reduce breast cancer deaths have mainly relied on early cancer detection and treatment. Recently, a total of 59 female patients with a diagnosis of breast cancer were enrolled and both UTR and U-II were immunohistochemically determined in tumour and normal tissues. U-II and UTR were expressed in 55 and 53 tumour samples, respectively, showing a strong positive correlation [65].

No statistically significant correlation was recorded between patient age and U-II [88] ($p=0.71$, $r= –0250$), but a statistically weak negative correlation was found between patient age and UTR ($p=0.038$, $r= –0281$). U-II and UTR were higher in the pre-menopausal patients. The only pathological characteristic found to be correlated with both high UTR and U-II expression was the absence of extranodal invasion in patients with lymph node metastases. U-II was significantly lower in patients with lymphatic invasion. Overall these findings suggest a role as good prognostic factor for both U-II and UTR in breast cancer even if studies on larger series of patients and correlation with survival data are strongly warranted to give definitive opinions [65]. In another recent report, the role of U-II plasma levels and of the U-II gene polymorphisms was studied in breast cancer patients [89].

One hundred forty-nine breast cancer patients and 148 healthy subjects with age-matched characteristics were enrolled and U-II plasma levels and Thr21Met and Ser89Asn polymorphisms in UST2 gene were detected [89]. The authors found a significant decrease of the circulating protein levels in patients compared with healthy controls.

Moreover, only Thr21Met polymorphism in UST2 gene was highly expressed in breast cancer patients with a statistically very high significance ($p= 0.0001$) [89]. As a whole, these data suggest that UTR and U-II expression in tumour tissues and U-II protein serum levels are good prognosticators in breast cancer, whereas the occurrence of Thr21Met mutation in U-II is a strong predictor of tumour occurrence. Since UT-II may act as an autocrine/paracrine factor and is able to regulate invasion and migration, which represent two important steps in carcinogenesis, it plays a key role in tumour biology of breast cancer. Particularly, Thr21Met polymorphism represents a risk factor because it increases breast cancer susceptibility, probably by affecting molecular mechanisms at the basis of the pathogenesis and development of breast cancer. This polymorphism could affect the binding efficiency of U-II to its receptor thus reducing its paracrine or autocrine effects.

Glioblastoma

Glioblastoma [glioblastoma multiforme (GBM)] is the most frequent and aggressive form of adult primary central nervous system tumor. Treatment of GBM patients with surgery, radiotherapy and chemotherapy improved median survival up-to 40–50 weeks. The combination of radiotherapy and temozolomide (TMZ) is recognized as the gold-standard first-line treatment for GBM [90, 91]. The promoter of the DNA repair gene O6-methylguanine-DNA methyltransferase resulted methylated in a group of patients with improved survival [92]. Despite the hard line therapy,tumors invariably relapse, median survival is 60-70 weeks and 5-years survival

Table 2. Urotensin-II Receptor involvement in neoplastic diseases.

Disease	Involvement Evidence	Drug	Ref.
Prostate Cancer	U-II controls proliferation, invasion and motility of prostate cancer cells; inverse correlation between UTR expression and Gleason grade.	Urantide	[60]
Prostate Cancer	UTR expression was a significant predictor of Gleason upgrading	ND	[74]
Bladder Cancer	UTR controls proliferation, invasion and motility of bladder cancer cells; correlation between UTR low expression and recurrence rate of NMIBC	UPG84	[62]
Hepatocellular Carcinoma	In mice association between pre-cancerous lesions induced by the exposure to DEN and the level of plasmatic and tissue UTR and U-II expression was found	ND	[63]
Hepatocellular Carcinoma	Higher U-II and UTR mRNA and protein expression in cancer tissue than in healthy tissues of the same patients; U-II increased phosphorylated PKC, ERK, and p38 MAPK and stimulated cell growth	ND	[64]
Colorectal Cancer	UTR regulates proliferation, motility and invasion of colorectal cancer cells; Inverse correlation between UTR expression and colorectal cancer grading *in vivo*	UPG83 and UPG85	[59]
Breast Cancer	U-II and UTR were expressed in 55 and 53 tumour samples, respectively. UTR and U-II expression correlated with absence of extranodal invasion.	ND	[65]
Breast Cancer	UTR and U-II expression in tumour tissues and U-II protein serum levels are good prognosticators, whereas the occurrence of Thr21Met mutation in U-II is a strong predictor of tumour onset.	ND	[56]
Glioblastoma	U-II behaves as a chemokine initiating directional cell migration through UT/G13 and Rho/ROCK	ND	[94]

U-II: Urotensin-II; UTR: Urotensin-II Receptor; ET-1: Endothelin-1; NMIBC: non-muscle invasive bladder cancer; DEN: Diethylnitrosamine; ERK: Extracellular-signal-regulated kinase; MAPK: Mitogen-activated protein kinase; PKC: Protein kinase C; ROCK: RhoA/Rho kinase; ND: not detected.

rate is less than 10%. Recently, it has been reported that UTR and Gamma-AminoButyric Acid Receptors (GABAAR) are coexpressed in rat glial cells, in glioma cells and in human astrocytes, and that U-II inhibited the repressor activity of GABAAR in rat astrocytes.

Moreover, UTR once activated, inhibited GABAAR function and induced its endocytosis in CHO and human astrocytes. This UII-mediated inhibition of the GABAergic activity may be involved in astrocyte growth and in glioma development [93]. U-II and its receptor have been recently studied in GBM and the authors found that intense U-II and UTR staining were detected in pseudopalissadic perinecrotic areas; moreover UII was also expressed in perivascular regions that were positive for CD34. UTR was found in fresh explants from 8 different resected GBM, in many cancer cells, in explanted cells and in vascular regions. UTR and U-II expression in human GBM cell lines suggested that UTR/U-II axis regulated tumor cells in auto/paracrine fashion. The authors demonstrated that U-II works as a chemotactic factor for GBM cells when a gradient of U-II concentration was used, whereas U-II alone had no effects on the migratory properties of tumour cells. Moreover, they demonstrated that U-II initiates cell migration by acting as chemokine and activating UT/ G13 and Rho/ROCK pathways, that include Gi/o and PhosphatidylInositol 3-Kinase (PI3K) components leading to Akt activation in GBM.

The signalings induced by U-II/UTR axis are different for actin polymerization or lamellipodia formation. In fact, upon binding of U-II, UTR recruits G13 and Rho/ROCK signaling cascade for actin polymerization, activates a Gi/PI3K pathway for lamellipodia formation and UT/Gi/o pathway is engaged for focal point formation [94]. Moreover, the same authors demonstrated that U-II, by binding UTR, was able to induce β-arrestin 1/2 and G protein-mediated (Gq, Gi, Go, G13) pathways, to trigger downstream pathways as ERK1/2 and to induce migration and adhesion in HEK cells [95]. U-II shows pleiotropic effects since its receptor couples to Gi, Go, Gq, and G13 and β-arrestins and it is involved in the pathogenesis of several tumours. In native and tumoral glial cells UT is closely associated to GABAAR. A cross-talk between UT and GABAAR could lead to the loss of the GABAAR expression in the plasma membrane. Moreover it would play a key role in the induction of cell proliferation by supporting transition from quiescent to proliferant astrocytes. Additionally, UT is able to act as a chemotaxis receptor and to activate specific transductional signals that are responsible for the switch between cell migration and adhesion by affecting molecular mechanisms involved in tumour invasion. For a summary of the evidence of UTR involvement in cancers (Table **2**).

Agonists and Antagonists Generation: How to Modulate UTR Function

Many design and development studies with agonists and antagonists of the octapeptide U-II(4-11) have been performed in order to either block or stimulate UTR and its downstream signal transduction pathways. The development of the potent agonist, P5U [96] and the antagonist, named

Urantide (Urotensin Antagonist Peptide) [97] are valuable accomplishments of this. The substitution of Cys5residue in the fragment of U-II(4-11) [98] with Pen produced P5U peptide, whose linear formula is the following: H-Asp-c[Pen-Phe-Trp-Lys-Tyr-Cys]-Val-OH. In experiments evaluating contractive capacity in rat aorta, P5U has shown an about 20-fold increase in potency compared to hU-II [96]. Optimization of this lead compound has been aimed to stabilize specific conformation and to improve pharmacokinetic properties. Consistently, by chemical modification occurred on P5U sequence, Carotenuto *et al.* have recently performed the synthesis of an agonist more potent than P5U, by the replacement of Tyr9 residue with a benzothiazolylalanine residue [99]. In previuos studies, P5U has been modified in positions 7 and 8, normally taken by Trp and Lys, respectively. The simulataneous substitution of Trp7 and Lys8 has driven to achievement of a potent antagonist, named Urantide, whose linear formula is: H-Asp-c[Pen-Phe-DTrp-Orn-Tyr-Cys]-Val-OH. Urantide has become the reference compound used to evaluate antagonist activity [100, 101]. Among the strategies aimed at antagonizing UTR effects, it has been recently described the possibility to benefit from allosteric modulators of the receptor such as Urocontrin and Urocontrin A. These have allowed to discriminate the effects derived from bindings of U-II or URP towards UTR due to the fact that they act as antagonists blocking the bond between U-II/URP and UTR through a non-competitive mechanism [102-104]. Although efforts to realize novel U-II derivatives have been successfully spent to provide more potent compounds, the candidacy of such peptides as drugs remains limited due to their unfavourable pharmacokinetic properties. Attempts to overcome the drawbacks of their therapeutic use have been offered by the development of nonpeptide or peptidomimetic molecules on the basis of pharmacophoric core sequence and secondary structure. Thus, different nonpeptide agonists (*e.g.* FL104, AC7954) and antagonists (*e.g.* Palosuran, SB-657510, GSK1562590 and GSK1440115) have been discovered and described in literature [105-108]. In general, all nonpeptide U-II analogues reported, normally preserve the three essential structural features, that is two aromatic rings and a basic group [109], which are necessary for the interaction with receptor counterpart. Moreover, pyrrolodiazepinones have been recently used as scaffolds to mimic the Bip-Lys-Tyr sequence of Urocontrin leading to new molecules able to selectively modulate U-II- and URP-mediated biological activity [110]. An alternative and efficient way to regulate the function of U-II/UTR axis is the downregulation and knock down of the receptor through the use of interference strategies. The delivery of siRNAs in tumour is an attractive and promising strategy that is becoming more feasible through the use of nanocarriers. Indeed, nanocarriers can allow the selective accumulation of the siRNA in tumours through the so-called Enhanced Permeation and Retention Effect (EPR).

CONCLUSION

U-II→UTR axis has been demonstrated to be involved in the regulation of blood pressure and blood pressure-correlated disease [111, 112], however strong evidence suggests its role in the regulation of proliferation and development of several epithelial cancers. For a large number of oncological diseases taken into consideration in this review, it is necessary to identify a prognostic marker that must be independent of histological analysis performed by a pathologist, influenced by his/her experience. This is important for the planning of more suitable therapies in relation to the degree of neoplastic disease. The involvement of UTR expression in tumour tissues in the prediction of the clinical outcome of the diseases alsosuggests its use as new prognostic factor in cancer illnesses.

The existence of specific modulators of UTR activity and the possible development of interference strategies make UTR as a possible new therapeutic target for the control of cancer diseases.

LIST OF ABBREVIATIONS

Akt	=	Protein kinase B
AM	=	Adrenomedullin
cAMP-PKA	=	Cyclic adenosine monophosphate-protein kinase A
CD	=	Cluster of differentiation
c-Src	=	Src kinase
DAG	=	Diacylglycerol
DEN	=	Diethylnitrosamine
EGFR	=	Epidermal growth factor receptor
EPR	=	Enhanced permeation and retention effect
ERK	=	Extracellular signal regulated kinase
ERK	=	Extracellular-signal-regulated kinase
ERS	=	Endoplasmic reticulum stress
ET-1	=	Endothelin-1
ETa	=	type a receptor endothelin
ETb	=	Type b receptor endothelin
FAK	=	Focal adhesion kinase
GABAAR	=	Gamma-aminobutyric acid receptors
GMB	=	Glioblastoma multiforme
GMC	=	glomerular mesangial cells
GPR	=	G protein coupled-receptor
GSK-3beta	=	Glycogen synthase kinase 3 beta
HCC	=	Human hepatocarcinoma
HuCC	=	Human corpus cavernosous
*h*U-II	=	Human Urotensin-II
ICP	=	Intracavernous pressure
IP_3	=	Inositol trisphosphate
LAM	=	Lymphangioleiomyomatosis
MAPK	=	Mitogen-activated protein kinase
MEK	=	Mitogen-activated protein kinase kinase
MIBC	=	muscle invasive bladder cancer
MLCK	=	Myosin light chain kinase
mPAP	=	Mean pulmonary arterial pressure

mTOR	=	Mammalian target of rapamycin
NADPH	=	nicotinamide adenosine dinucleotide phosphate
NHE-1	=	Sodium/hydrogen exchanger 1
NMIBC	=	non-muscle invasive bladder cancer
PAH	=	Pulmonary arterial hypertension
PI3K	=	PhosphatidylInositol 3-Kinase
PKC	=	Protein kinase C
PLC	=	Phospholipase C
RCC	=	Rat corpus cavernosous
RGS2	=	Regulator of G-protein signaling 2
ROCK	=	RhoA/Rho kinase
RT-PCR	=	Real time polymerase chain reaction
SEM	=	Scanning electron microscope
shRNA	=	Short hairpin RNA
SOCE	=	Store-operated Ca(2+) entry
SST-14	=	Somatostatin-14
TMZ	=	Temozolomide
U-II	=	Urotensin-II
UIIKO	=	UII gene deletion in mice
URP	=	Urotensin-II related peptide
UTR	=	Urotensin-II receptor

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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