Chapter 3
Apoptosis and Autophagy

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Abstract Defects in both apoptotic and non-apoptotic cell-death pathways are strictly associated with tumorigenesis. In particular, resistance to apoptosis is considered to be an hallmark of cancer cells. Defects in apoptosis underlie not only tumorigenesis, but also resistance to cancer treatments.

A better definition of non-apoptotic and apoptotic cell-death pathways interactions is needed. Since the first attempts of cell deaths classification, the caspase-dependent, tolerogenic, programmed and physiological cell death instances have been contrasted to their caspase-independent, immunogenic, accidental and pathological counterparts. However, further investigation of non-apoptotic pathways might provide new therapeutic strategies aimed at inducing the non-apoptotic death of cancer cells.

In the present chapter, apoptotic and non-apoptotic cell death pathways are discussed for what concern neoplastic transformation of prostate gland.

As most human neoplastic diseases, prostate cancers (most of them are adenocarcinomas) develop when the rates of cell division and cell death are no longer equal, leading to uncontrolled tumor growth. Following the initial transformation event, further mutations of a multitude of genes can lead to tumor progression and metastasis. To date, several molecular signalling pathways have been found altered in prostate cancer, such as, to cite some, the Androgen and Estrogen metabolism, the cell cycle progression control, the MAPK signalling pathway, the maintenance of the stability of the genome, the control of Apoptosis and Autophagy.

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Both apoptosis and autophagy are stress response mechanisms that have been involved in neoplastic transformation of prostatic gland and that seem to be the most affected especially in the latter stages of prostate cancer progression.

Apoptosis, the most common and well-defined form of programmed cell death (PCD), is a crucial cellular process in normal and pathological conditions: its importance during embryonic development and the maintenance of tissue homeostasis of multicellular organisms has been assessed long ago (Meier et al. 2000). Dysregulation of apoptosis has been implicated in numerous pathological conditions, including neurodegenerative diseases and autoimmunity, moreover its dysfunction is *de facto* accepted as an hallmark of cancer (Hanahan and Weinberg 2000, 2011).

In mammalian cells, the apoptotic process is mediated by a family of cysteine proteases known as the caspases (Alnemri et al. 1996). To keep the apoptotic programme under control, caspases are initially expressed in cells as inactive procaspase precursors. When initiator caspases—such as caspase-8 and caspase-9—are activated by oligomerization, they cleave the precursor forms of effector caspases, such as caspase-3, caspase-6 and caspase-7 (Salvesen and Dixit 1997; Cryns and Yuan 1998; Thornberry and Lazebnik 1998). Activated effector caspases in turn cleave a specific set of cellular substrates, resulting in the well-known constellation of biochemical and morphological changes that are associated with the apoptotic phenotype.

Autophagy is a process in which subcellular membranes undergo dynamic morphological changes that lead to the degradation of cellular proteins and cytoplasmic organelles. This process is an important physiological cellular response to stress or starvation. Many studies have shed light on the involvement of autophagy in cancer, but it is still unclear whether autophagy suppresses tumorigenesis or provides cancer cells with a rescue mechanism under unfavourable conditions. In fact, while apoptosis is clearly a primary cell death mechanism, there is much controversy about the functional role of autophagy in life and death. Depending on the cellular context, the cell line and the stimulus, autophagy either favours or counteracts cell death signalling.

It is believed that multiple connections exist between autophagy and apoptosis, and so the molecular interplay and functional relationship between their pathways have gained considerable interest in normal and neoplastic condition.

In the present chapter, a review of recent literature about the strict relationship between apoptosis, autophagy and prostate cancer is reported, with major emphasis on the role of deregulated apoptosis and autophagy during prostate cancer progression and the therapeutic strategies based on these cellular processes.

3.1 The Biology of Prostate Cancer

Prostate cancer is generally regarded as multifocal, since primary tumors often contain multiple independent histologic foci of cancer, that are often genetically distinct (Aihara et al. 1994; Bostwick et al. 1998; Macintosh et al. 1998; Mehra et al.

2007; Clark et al. 2008). In contrast, molecular and cytogenetic analyses show that multiple metastases in the same patient are clonally related, indicating that advanced prostate cancer is monoclonal (Mehra et al. 2008; Liu et al. 2009).

The prostate gland can be the site of multiple neoplastic transformation events, many of which give rise only to latent prostate cancer that does not progress to clinically detectable disease.

It is widely accepted that PIN represents a precursor for prostate cancer (Bostwick 1989; DeMarzo et al. 2003). PIN is generally characterized at the histological level by the appearance of luminal epithelial hyperplasia, reduction in basal cells, enlargement of nuclei and nucleoli, cytoplasmic hyperchromasia, and nuclear atypia; in addition, high-grade PIN lesions generally display marked elevation of cellular proliferation markers (Bostwick 1989; Shappell et al. 2004). In contrast with prostate cancer, however, basal cells are reduced in number in PIN, but are not absent.

While evidence of major subtypes of prostate cancer is lacking at the histopathological level, recent genomic analyses have provided increasing evidence for molecularly defined subtypes (Tomlins et al. 2008; Palanisamy et al. 2010; Taylor et al. 2010). In particular, expression profiling analyses of prostate cancer specimens have not strictly defined molecular signatures associated with distinct cancer subtypes that specifically correlate with disease outcome (Singh et al. 2002; Lapointe et al. 2004; Tomlins et al. 2007). However, oncogenomic pathway analyses that integrate analyses of gene expression, copy number alterations, and exon resequencing may provide a unified approach for distinguishing prostate cancer subtypes and stratifying patient outcome (Taylor et al. 2010).

Although common sites of secondary metastasis for prostate cancer are lung, liver, and pleura, if prostate cancer metastasizes, it invariably goes to bone, where it forms characteristic osteoblastic lesions (Bubendorf et al. 2000; Logothetis and Lin 2005).

The identification of key molecular alterations in prostate-cancer cells implicates carcinogen defenses (GSTP1), growth-factor-signaling pathways (NKX3.1, PTEN, and p27), and androgens (AR) as critical determinants of the phenotype of prostate-cancer cells. NKX3.1, PTEN, and p27 regulate the growth and survival of prostate cells in the normal prostate. Inadequate levels of PTEN and NKX3.1 lead to a reduction in p27 levels and to increased proliferation and decreased apoptosis. Androgen receptor (AR) is a transcription factor that is normally activated by its androgen ligand. During androgen withdrawal therapy, the AR signal transduction pathway also could be activated by amplification of the AR gene, by AR gene mutations, or by altered activity of AR coactivators. Through these mechanisms, tumor cells lead to the emergence of androgen-independent prostate cancer.

In order to elucidate the relationship between prostate cancer, apoptosis and autophagy, we will focus on the following genes and signalling pathways, often found involved in these tumors:

- *PTEN*
- *AKT/mTOR and MAPK signalling pathways*

- *p53*
- *Bcl2*
- *Beclin I*

3.1.1 PTEN

PTEN (Tumor suppressors phosphatase and tensin-homolog deleted on chromosome 10) was originally identified as a tumor suppressor, is frequently mutated or deleted in many cancers, including prostate (Salmena et al. 2008). The relevance of PTEN loss for prostate cancer was initially inferred from its location on chromosomal region 10q23, which frequently undergoes allelic loss in prostate cancer, as well as by its reduction or loss of expression in prostate tumors (Wang et al. 1998; Whang et al. 1998; McMenamin et al. 1999; Dong et al. 2007). Earlier studies had generated conflicting data regarding whether both alleles of PTEN are deleted in prostate cancer, or, if one allele is deleted, whether the remaining allele is mutated, or if the expression of PTEN protein is reduced, inactivated, or altered in subcellular localization. To resolve these issues, recent studies have investigated PTEN copy number, mutational status, and/or protein expression in primary or castration-resistant tumors using multiple experimental approaches (Verhagen et al. 2006; Schmitz et al. 2007; Sircar et al. 2009; Taylor et al. 2010). In combination with the consensus of previous reports, these studies support the conclusion that PTEN undergoes copy number loss as an early event in prostate carcinogenesis, and is correlated with progression to aggressive, castration-resistant disease. Interestingly, these studies have also suggested that low levels of PTEN activity may be retained in prostate cancer—an observation that parallels the haploinsufficiency of NKX3.1 and the p27 cell cycle regulator (Gao et al. 2004; Abate-Shen et al. 2008), and which may reflect the relative indolence of prostate tumors.

Analyses of Pten deletion in genetically engineered mouse models have uncovered its cooperativity with inactivation of other key genes that are deregulated in prostate tumorigenesis, and have also provided insights into new therapeutic options for the treatment of prostate cancer. Germline loss of Pten in heterozygous mutants or conditional deletion in the prostate epithelium results in PIN and/or adenocarcinoma (Di Cristofano et al. 1998; Podsypanina et al. 1999; Trotman et al. 2003; Wang et al. 2003). Inactivation of Pten has been shown to cooperate with loss of function of the Nkx3.1 homeobox gene, up-regulation of the c-Myc proto-oncogene, or the TMPRSS-ERG fusion (Kim et al. 2002, 2009; Carver et al. 2009; King et al. 2009). Notably, PTEN reduction or loss in prostate cancer predisposes to the emergence of castration-resistant prostate cancer (Mulholland et al. 2006; Shen and Abate-Shen 2007). In particular, perturbation of PTEN expression in human prostate cancer cell lines or targeted deletion of Pten in mouse prostate cancers is sufficient for the development of castration resistance (Lin et al. 2004; Bertram et al. 2006; Gao et al. 2006; Wu et al. 2006). While this may reflect the ability of PTEN to interact directly with AR, the mechanistic details by which PTEN loss promotes castration resistance remain to be resolved.

3.1.2 Akt/mTOR and MAPK Signalling Pathways

Constitutive activation of the PI3K/AKT/mTOR axis is a survival mechanism commonly encountered in human cancer. The abnormal activation of this pathway can be ascribed to diverse cellular events, such as loss of PTEN, loss of tuberous sclerosis complex (TSC) 1 and 2, amplification or mutation of class I PI3K, overexpression of AKT, constitutive activation of tyrosine kinase growth factor receptors and exposure to carcinogens. In prostate cancer, the up-regulation of the Akt/mTOR signaling pathway has been mainly ascribed to the loss of function of Pten gene, primarily through activation of Akt1 (Thomas et al. 2004; Chen et al. 2006b; Mulholland et al. 2006; Shen and Abate-Shen 2007). Nevertheless, up-regulation of this pathway in prostate cancer can also take place through activating mutations of Akt1 (Boormans et al. 2008), or through activation of the p110b isoform of PI3K (Hill et al. 2010; Lee et al. 2010). The functional consequences of Akt/mTOR pathway activation are particularly relevant for castration-resistant prostate cancer, as has been shown in genetically engineered mouse models, in gain-of-function studies with orthotopic grafting or tissue recombination models, as well as in human cell lines (Majumder et al. 2003; Uzgare and Isaacs 2004; Gao et al. 2006; Xin et al. 2006). The consequences of Akt activation are mediated in part by activation of NF-kB signaling via stimulation of IKK (Dan et al. 2008). Conversely, functional studies in mouse models and correlative studies in human prostate cancer have implicated deregulated NF-kB signaling in mediating androgen responsivity, metastasis, and disease outcome (Fradet et al. 2004; Ismail et al. 2004; Lessard et al. 2006; Luo et al. 2007; Zhang et al. 2009).

Constitutive activation of the PI3K/AKT/mTOR axis result in autophagy suppression; the relationship between the PI3K/AKT/mTOR pathway and autophagy is also suggested by the findings that G-protein coupled receptor (GPCR) antagonists to growth factor receptors (GFR), class I PI3K inhibitors such as lithium and carbamazepine, AKT inhibitors such as perifostine and AKT/PKB signaling inhibitor-2 (API-2), and mTOR inhibitors such as rapamycin, RAD-001 and CCI-779, result in autophagy induction (Nicholson and Anderson 2002; Majumder and Sellers 2005; Moretti et al. 2007).

Tumors with high metabolic demands, such as those with constitutively active PI3K mutations, PTEN loss or AKT activation, would be expected to be dependent on autophagy for energy homeostasis and survival. Thus, suppression of autophagy by the PI3K signaling cascade presents a disadvantage that these rapidly proliferating tumor cells may have to overcome to remain viable, and leads to the prediction that compensatory mechanisms, such as deregulated apoptosis and/or metabolism, may be concurrently activated to counteract the negative implications of defective autophagy on tumor cell survival.

In addition to Akt/mTOR signaling, Erk (p42/44) MAPK signaling is also frequently activated in prostate cancer, particularly in advanced disease, and is often coordinately deregulated together with Akt signaling (Abreu-Martin et al. 1999; Gioeli et al. 1999; Paweletz et al. 2001; Malik et al. 2002; Thomas et al. 2004; Kinkade et al. 2008). The mitogen-activated protein kinases (MAPKs) are the

family of kinases that transduce signals from the cell membrane to the nucleus in response to a wide range of stimuli, including stress. MAPKs are serine/threonine kinases that, upon stimulation, phosphorylate their specific substrates at serine and/or threonine residues. Such phosphorylation events can either positively or negatively regulate substrate, and thus entire signalling cascade activity. Thus, the MAPK signalling pathways modulate gene expression, mitosis, proliferation, motility, metabolism, and programmed cell death 'apoptosis'. It has been demonstrated that constitutive activation of the MAPK ERK regulates the maturation of autophagosomes (Corcelle et al. 2006); moreover, the oncogenic activation of ras (rasV12), the upstream activator of ERK, has been also reported to induce autophagic vacuolation (Chi et al. 1999; Pattingre et al. 2003).

Simultaneous activation of Akt/mTOR and Erk (p42/44) MAPK signalling pathways promotes tumor progression and castration resistance in prostate cancer cell lines and mouse models (Uzgare and Isaacs 2004; Gao et al. 2006), while combinatorial inhibition of these pathways inhibits castration-resistant prostate cancer in genetically engineered mice (Kinkade et al. 2008). In contrast with Akt/mTOR signalling, the upstream events that lead to activation of Erk MAPK signaling are less well defined, but are thought to be linked to aberrant growth factor signaling (Gioeli 2005). Although mutations of RAS or RAF are rarely found in human prostate cancer, the pathway is frequently perturbed in advanced prostate cancers (Taylor et al. 2010). Notably, expression of activated forms of either Raf or Ras in the mouse prostrate epithelium results in MAPK activation and promotes cancer formation (Jeong et al. 2008; Pearson et al. 2009).

3.1.3 p53

p53 (also known as TP53, for tumor protein p53), is a tumor suppressor protein that is encoded by the *TP53* gene. p53 is crucial in multicellular organisms, where it regulates the cell cycle and functions as a tumor suppressor. Because of its role in conserving genome stability by preventing accumulation of mutations, p53 has been also described as "the guardian of the genome".

p53 plays many roles in anticancer function; among them:

- It can activate DNA repair proteins when DNA has sustained damage.
- It can induce growth arrest by holding the cell cycle at the G_1/S regulation point on DNA damage recognition (allowing for the DNA repair proteins to fix the damage, so to permit the cell to continue the cell cycle).
- It can initiate apoptosis, the programmed cell death, if DNA damage proves to be irreparable.

In unperturbed conditions, the p53 protein is continually produced and degraded in the cell. The degradation of the p53 protein is associated with MDM2 binding. In a negative feedback loop, MDM2 is itself induced by the p53 protein. However, mutant p53 proteins often do not induce MDM2, and are thus able to accumulate at

very high concentrations. Worse, mutant p53 protein itself can inhibit normal p53 protein levels.

Patients carrying germline mutations of the *TP53* gene are most likely develop tumors in early adulthood, a disease known as Li-Fraumeni syndrome. Somatic mutations also occurs with a very high rate: more than 50 % of human tumors, in fact, hold p53 mutations (Hollstein et al. 1991).

In prostate cancer, the frequency of p53 mutations seems to be lower than in other cancers. A relatively minor role for p53 in prostate carcinogenesis is consistent with the observation that Li-Fraumeni patients have a low incidence of prostate cancer (Kleihues et al. 1997), although it has been hypothesize that they may die by other carcinomas before they can develop prostate cancer.

Loss of chromosome 17p occurs in advanced stages of prostate cancer and metastatic disease (Cher and Carroll 1994; Cher et al. 1996; Saric et al. 1999), deleting a region that includes the p53 locus, but not BRCA1 (Brooks et al. 1996). It is now generally accepted that mutations of p53 occur infrequently in early invasive carcinoma (Henke et al. 1994; Voeller et al. 1994; Prendergast et al. 1996). In contrast, p53 is mutated in advanced stages of prostate cancer, as well as in recurrent and metastatic disease (Effert et al. 1993; Navone et al. 1993; Aprikian et al. 1994; Eastham et al. 1995; Heidenberg et al. 1995). Moreover, several studies indicate that p53 overexpression is a predictive factor for poor prognosis and disease recurrence, particularly when detected in combination with Bcl2 (Thomas et al. 1993; Shurbaji et al. 1995; Bauer et al. 1996; Moul et al. 1996; Matsushima et al. 1997; Theodorescu et al. 1997; Brewster et al. 1999; Stackhouse et al. 1999).

p53 appears to have a dual role in autophagy regulation. Upon DNA damage, hypoxia and oncogene activation, p53 has been shown to transactivate autophagy-inducing genes and stimulate autophagy by inhibiting mTOR in an AMP-activated protein kinase (AMPK)- and TSC1/TSC2-dependent manner. p53 also induces autophagy via its direct target damage-regulated autophagy modulator (DRAM). At the same time, however, genetic or pharmacologic inactivation of cytoplasmic p53 also triggers autophagy, indicating that the non-nuclear p53 pool is a potent autophagy repressor (Jones et al. 2005; Budanov and Karin 2008; Maiuri et al. 2009a, b; Feng and Levine 2010). Thus, autophagy is activated as a stress-mitigating mechanism by both stress-mediated p53 induction and stress-exacerbating p53 loss. The circumstances and the molecular pathways involved in the decision to use p53 for autophagy activation versus inhibition in cancer cells have not yet been determined. Plausibly, p53 loss, and thus autophagy induction, or negative regulation of autophagy inhibition, may be one of the compensatory mechanisms that tumor cells use to counter-balance the survival-undermining effects of autophagy suppression by an activated PI3K/AKT/mTOR axis.

3.1.4 Bcl2

Bcl-2 (B-cell lymphoma 2) is the founding member of the Bcl-2 family of apoptosis regulator proteins encoded by the BCL2 gene (Tsujimoto et al. 1984; Cleary et al. 1986).

So far, 15 mammalian family members were identified, which were divided into three subfamilies:

1. Bcl-2 subfamily (pro-survival): ***Bcl-2***, Bcl-XL, Bcl-w, Mcl-1 and A1;
2. Bax subfamily (pro-apoptotic): Bax, Bak and Bok;
3. BH3 subfamily (pro-apoptotic): Bad, Bid, Bik, Blk, Hrk, BNIP3 and BimL;

Additionally, several Bcl-2 homologs have been identified in viruses, among others the adenovirus oncoprotein E1B-19 K.

A central checkpoint of apoptosis is the activation of Caspase-9 by mitochondria. Bcl-2, and Bcl-XL, can bind to the C terminal part of Apaf-1 (to the CED-4 like part and the WD-40 domain), thus inhibiting the association of Caspase-9 with Apaf-1. The pro-survival proteins also seem to maintain organelle integrity since Bcl-2 directly or indirectly prevents the release of cytochrome c from mitochondria.

Overexpression of Bcl2 in prostate carcinoma cells is a hallmark of advanced, hormone-refractory disease, and may account for the resistance to apoptosis that is characteristic of late stages (Colombel et al. 1993; McDonnell et al. 1997). Although Bcl2 expression is restricted to basal cells in the normal prostate, forced expression of Bcl2 in LnCAP prostate carcinoma cells protects against apoptosis induced by androgen depletion (Raffo et al. 1995). Moreover, as is the case for p53, Bcl2 expression may provide a prognostic marker that correlates with disease outcome (Mackey et al. 1998). Indeed, several preliminary studies have examined whether Bcl2 inactivation may prevent tumor recurrence (Miyake et al. 1999). Overexpression of Bcl2 has been shown to confer resistance to chemotherapy in prostate carcinoma cell lines (Tu et al. 1995), and current clinical efforts are aimed at modulating the expression of Bcl2 (DiPaola and Aisner 1999).

3.1.5 Beclin-1 and the Crosstalk Between Apoptosis and Autophagy

Apoptosis and autophagy share similarities in that both are self-degradative cellular pathways activated under conditions of stress.

The potential for crosstalk between apoptosis and autophagy was first recognized when Beclin 1 was initially identified as a Bcl-2-interacting protein. Regulators of apoptosis, such as Bcl-2/Bcl-xL and the BH3-only proteins, interact with Beclin 1 and can modulate autophagy (Wang 2008). The anti-apoptotic protein Bcl-2 binds to Beclin 1 under non-stress conditions and inhibits autophagy in the ER, whereas the BH3-only protein Bad, BNIP3, and BH3 mimetics, such as ABT737, competitively inhibit the interaction between Beclin 1 and Bcl-2/BclxL and stimulate autophagy (Wang 2008). We can conclude that, up to our knowledge, positive regulators of apoptosis also induce autophagy, which is reasonable given that both pathways are activated under similar stress conditions. The cell fate, in response to metabolic stress, is determined by the functional status and the interaction between the stress-mitigating pathways of apoptosis and autophagy.

In prostate cancer some data are available from cultured cell lines experiments. It has been recently demonstrated that in PC3, prostate cancer cell lines, the Ursolic Acid-induced autophagy is mediated through the Beclin-1 and Akt/mTOR pathways. (Ursolic acid is a pentacyclic triterpenoid, that inhibit the growth of cancer cells by cell cycle arrest and the stimulation of apoptosis). Inhibition of autophagy by either 3-methyladenine or Beclin-1/Atg5 small interfering RNA enhanced UA-induced apoptosis (Shin et al. 2012). Moreover, it has been shown that autophagy is elevated in LNCaP cells under androgen deprivation conditions, which results in increased cell viability (Li et al. 2008) (Fig. 3.1).

3.2 Non-apoptotic/Non-autophagic Cell Death Pathways in Prostate Cancer

3.2.1 Anoikis

Anoikis (from a Greek word meaning "homelessness") is defined as anchorage-dependent programmed cell death. It can be considered an apoptotic process that is induced by inadequate or inappropriate cell–matrix interactions. Anokis is used to describe the apoptotic response elicited by the absence of cell-matrix interactions (Frisch and Screaton 2001).

In prostate carcinoma cell lines, anoikis has been reported to be regulated by Bcl2-independent pathways; mitochondrial DNA depletion in prostate epithelial cells promotes anoikis resistance and invasion through activation of PI3K/Akt2. Several papers propose Anoikis as a novel therapeutic target for prostate cancer (Bondar and McConkey 2002; Garrison and Kyprianou 2004; Hasanuzzaman et al. 2007; Moro et al. 2009; Sakamoto and Kyprianou 2010).

3.2.2 Autoschizis

Autoschizis is a term derived from the Greek roots "auto" meaning self, and "skhizein" to split. It indicates a recently described form of cancer cell death characterized by a reduction in cell size due to the loss of cytoplasm through self-excision. This process occurs without cell organelles loss, in absence of morphologic degradation of the cells nucleus and nucleolus and without the formation of apoptotic bodies and destruction of the cell membrane. The cell death results from karyorrhexis and karyolysis. Autoschizis can be initiated via in vivo treatment with Vitamin C (VC), synthetic Vitamin K (VK3) or a combination of both. The treatment has been tested on various types of cancers with positive results (Jamison et al. 2002)

A combination of vitamin C/K(3) has been reported to induce cell death by autoschizis in prostate carcinoma cell lines (Taper et al. 2001; Lasalvia-Prisco et al. 2003; Gilloteaux et al. 2005; Tomasetti et al. 2010).

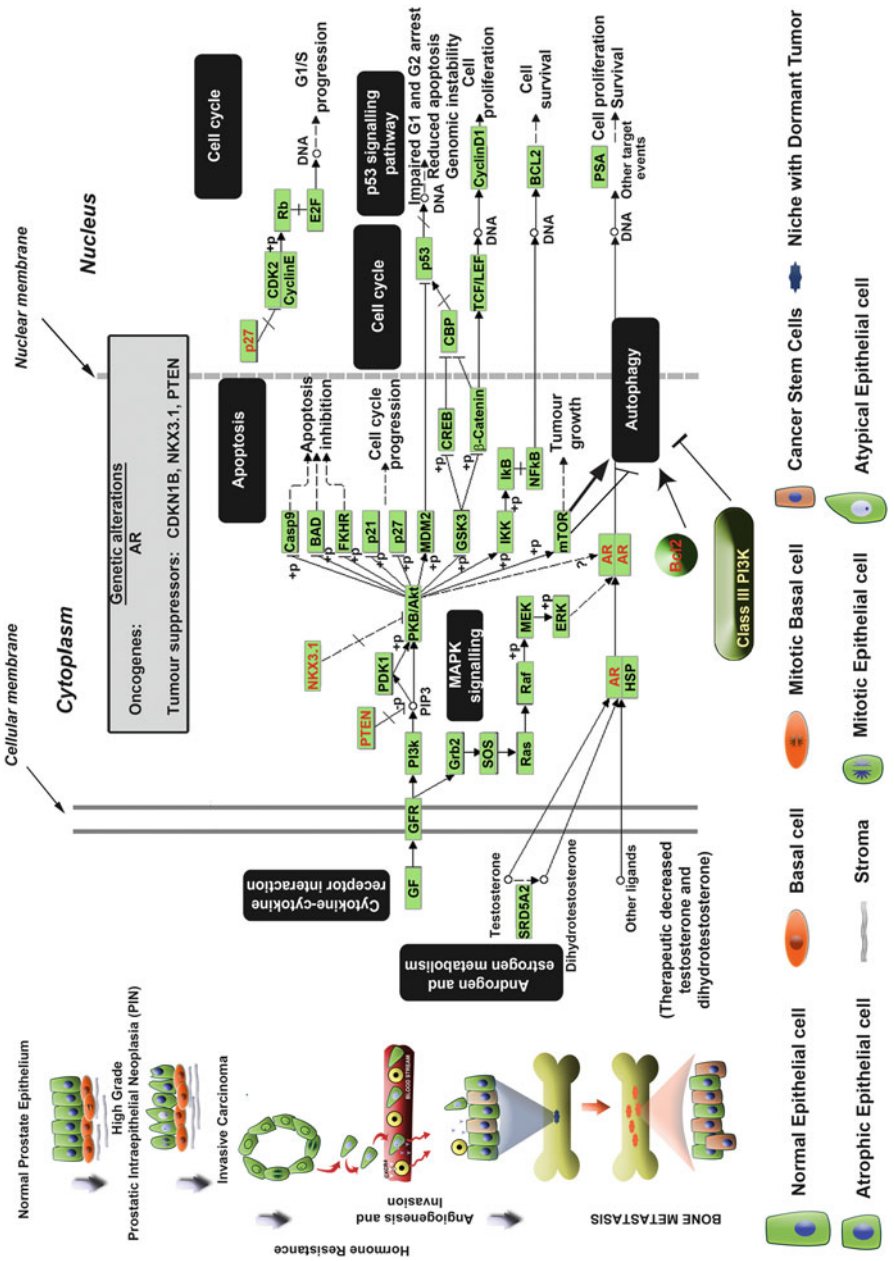
Normal Prostate Epithelium
High Grade Prostatic Intraepithelial Neoplasia (PIN)
Invasive Carcinoma
Hormone Resistance
Angiogenesis and Invasion
BONE METASTASIS
Cellular membrane
Cytoplasm
Nucleus
Nuclear membrane
Genetic alterations
Oncogenes: AR
Tumour suppressors: CDKN1B, NKX3.1, PTEN
Cytokine-cytokine receptor interaction
Androgen and estrogen metabolism
Testosterone
Dihydrotestosterone
Other ligands
(Therapeutic decreased testosterone and dihydrotestosterone)
GF
GFR
Grb2
SOS
Ras
Raf
MEK
ERK
PI3k
PIP3
PDK1
PKB/Akt
PTEN
NKX3.1
MAPK signalling
SRD5A2
AR
HSP
Apoptosis
Casp9
BAD
FKHR
p21
p27
Apoptosis inhibition
Cell cycle progression
Cell cycle
p27
CDK2 CyclinE
Rb
E2F
DNA
G1/S progression
Cell cycle
p53
MDM2
CREB
CBP
p53 signalling pathway
Impaired G1 and G2 arrest
Reduced apoptosis
Genomic instability
β-Catenin
GSK3
IKK
IkB
NFkB
Tumour growth
TCF/LEF
CyclinD1
Cell proliferation
mTOR
Autophagy
BCL2
Cell survival
PSA
Cell proliferation
Survival
Other target events
Bcl2
Class III PI3K
Normal Epithelial cell
Basal cell
Mitotic Basal cell
Cancer Stem Cells
Niche with Dormant Tumor
Atrophic Epithelial cell
Stroma
Mitotic Epithelial cell
Atypical Epithelial cell

Fig. 3.1 Mechanism of cellular death in the genesis and progression of PCa. Apoptosis and autophagy are both self-degradative cellular pathways activated under conditions of stress to eliminate unwanted or irreparably damaged cells and as a stress adaptive response to prolong cell survival, respectively. In this figure is reported the complex ridge of molecular pathway activated in cancerogenesis and progression of PCa, in which are comprised those regulating apoptosis and autophagy.
Loss of PTEN is an early event in prostate carcinogenesis and correlates with progression to castration-resistant disease and parallels with loss of function of the Nkx3.1 homeobox gene, up-regulation of the c-Myc proto-oncogene, or the TMPRSS-ERG fusion, involved in cellular death. Moreover, loss of function of PTEN causes the abnormal activation of the PI3K/AKT/mTOR axis and result in autophagy suppression. The latter event leads to compensatory mechanisms, such as deregulated apoptosis and/or metabolis to counteract the negative implications of defective autophagy on tumor cell survival.
The constitutive activation of the MAPK ERK regulates the maturation of autophagosomes and induce autophagic vacuolation.
p53 is a tumor suppressor protein that regulates the cell cycle and functions as a tumor suppressor. It regulate autophagy in a double manner: by inhibiting mTOR in an AMP-activated protein kinase (AMPK)- and TSC1/TSC2-dependent manner and via its direct target damage-regulated autophagy modulator (DRAM).
Bcl-2 inhibits the association of Caspase-9 with Apaf-1, and, therefore, its activation, so regulating negatively the apoptotic mechanism.
A potential for crosstalk between apoptosis and autophagy is hypothesizable because Beclin 1 is a Bcl-2-interacting protein and can modulate autophagy. The anti-apoptotic protein Bcl-2 binds to Beclin 1 under non-stress conditions and inhibits autophagy in the ER

3.2.3 Entosis

Entosis is a form of cell death that occurs when a cell dyes being engulfed by a neighboring cell. The process was discovered by Overholtzer, et al. as reported in *Cell* (Overholtzer et al. 2007).

Several works indicate entosis as a non-genetic cause of aneuploidy. Aneuploidy is common in human tumors and is often indicative of aggressive disease. Aneuploidy can result from cytokinesis failure, which produces binucleate cells that generate aneuploid offspring with subsequent divisions. In cancers, disruption of cytokinesis is known to result from genetic perturbations to mitotic pathways or checkpoints. It has been described a non-genetic mechanism of cytokinesis failure that occurs as a direct result of cell-in-cell formation by entosis. Live cells internalized by entosis, which can persist through the cell cycle of host cells, disrupt formation of the contractile ring during host cell division. As a result, cytokinesis frequently fails, generating binucleate cells that produce aneuploid cell lineages (White 2007; Janssen and Medema 2011; Krajcovic et al. 2011).

3.2.4 Excitotoxicity

The overactivation of receptors for the excitatory neurotransmitter glutamate (glutamate receptors) such as the NMDA receptor and AMPA receptor can lead to the so called Excitotoxicity, that is the pathological process by which nerve cells are damaged and killed by excessive stimulation.

Excitotoxins like NMDA and kainic acid which bind to these receptors, as well as pathologically high levels of glutamate, can cause excitotoxicity by allowing high levels of calcium ions (Ca2+) to enter the cell. Ca2+ influx into cells activates a number of enzymes, including phospholipases, endonucleases, and proteases such as calpain. These enzymes go on to damage cell structures such as components of the cytoskeleton, membrane, and DNA.

Glutamate carboxypeptidase II (GCPII) is a membrane responsible for the cleavage of N-acetyl-L-aspartyl-L-glutamate (NAAG) yielding free glutamate in the synaptic cleft, and is implicated in various pathologic conditions associated with glutamate excitotoxicity. The prostate form of GCPII, termed prostate-specific membrane antigen (PSMA), is up-regulated in cancer and used as an effective prostate cancer marker (Barinka et al. 2004; Ding et al. 2007).

3.2.5 Mitotic Catastrophe

Mitotic catastrophe is an event in which a cell is destroyed during mitosis. This is believed by some to occur as a result of an attempt at aberrant chromosome

segregation early in mitosis, or as a result of DNA damage later. Cells which fail to go through a mitotic catastrophe after a mitotic failure are likely to create aneuploid cells when they later reproduce, posing a risk of oncogenesis, potentially leading to cancer.

Mitotic catastrophe thus may be conceived as a molecular device that prevents aneuploidization, which may participate in oncogenesis. Mitotic catastrophe is controlled by numerous molecular players, in particular, cell-cycle-specific kinases (such as the cyclin B1-dependent kinase Cdk1, polo-like kinases and Aurora kinases), cell-cycle checkpoint proteins, survivin, p53, caspases and members of the Bcl-2 family (Castedo et al. 2004).

A large body of works show the correlation between mitotic catastrophe and prostate cancer. Taxols, such as docetaxel, has been shown to induce cell death by mitotic catastrophe, in prostate cancer cells, by concomitant activation of caspase and lysosomal pathways; resistance to docetaxel has been proven as a complex mechanism involving several genes, as shown by several genomic and proteomic approaches (Fabbri et al. 2008; Mediavilla-Varela et al. 2009; Balasubramani et al. 2011; Desarnaud et al. 2011).

3.2.6 Necrosis and Oncosis

Necrosis does not indicate a form of cell death but refers to changes secondary to cell death by any mechanism, including apoptosis. The term oncosis (derived from ónkos, meaning swelling) was proposed in 1910 by von Reckling-hausen precisely to mean cell death with swelling. Oncosis leads to necrosis with karyolysis and stands in contrast to apoptosis, which leads to necrosis with karyorhexis and cell shrinkage (Majno and Joris 1995).

Some compounds, such as Kahalalide F, a marine-derived compound, have been reported to induce oncosis in human prostate cancer cells (Suarez et al. 2003).

3.2.7 Paraptosis

Paraptosis, which has been observed in a variety of cell types in response to insulin derived growth factor 1 receptor, differs from apoptosis because of the lack of fragmentation of the cell, its nucleus, and its DNA, and from necrosis due to its requirement for new RNA and protein synthesis.

Paraptosis, like apoptosis, does indeed involve a caspase, caspase-9. Compounds able to modulate the proteasome along with Hsp90 protein, were also able to induce prostate carcinoma cell lines death by paraptosis (Wang et al. 2012).

3.3 Other Cell Death Pathways

3.3.1 Parthanatos

Parthanatos is a form of cell death that often occurs as a result of ischemia reperfusion injury. This form of cell death is distinct from apoptosis, necrosis, or autophagy and is being referred to also as PARP1-dependent cell death [poly(ADP-ribose) polymerase 1-dependent cell death]. Although it shows some features of cell death by apoptosis, it is not associated with the formation of apoptotic bodies. Parthanatos also differs from autophagy in that it does not involve the formation of autophagic vacuoles and lysosomal degradation (David et al. 2009).

3.4 Therapeutic Implications

The requirement of the mTORC2 complex as well as the p110b isoform of PI3K for tumor formation following Pten loss suggests that these signaling components may provide additional and/or alternative targets for therapeutic intervention (Jia et al. 2008; Guertin et al. 2009). Moreover, the observation that complete inactivation of Pten in mouse prostate tumors leads to cellular senescence (Chen et al. 2006a) has led to the idea that novel therapeutic approaches might promote senescence for selective targeting of prostate tumor cells through knockdown of Pten function (Alimonti et al. 2010) or targeting of Skp2 (Lin et al. 2010). Furthermore, a small percentage of aggressive prostate tumors contains a translocation of B-RAF or C-RAF that results in activation (Palanisamy et al. 2010). This let envisage a further therapeutical approach based on the pharmacological inhibition of RAF kinase.

Finally, several novel prostate cancer cells killing strategies are based on different cell death pathways, especially apoptosis and autophagy, that have been proved to be an interesting field of investigation in order to find efficient therapeutic approaches for androgen-resistant and metastatic prostate carcinomas.

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