We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Genetic Instability in Normal-Appearing and Tumor Urothelium Cells and the Role of the TP53 Gene in the Toxicogenomic Effects of Antineoplastic Drugs

Daisy Maria Favero Salvadori and
Glenda Nicioli da Silva

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/53502>

1. Introduction

Bladder cancer is one of the most common urinary neoplasms in industrialized countries, with more than 50,000 new cases diagnosed annually in Europe and North America [1,2]. In most countries of the Western world, transitional cell carcinomas (TCCs) account for 90% of the malignancies of this organ, while 5% are identified as squamous cell carcinomas and 2% as adenocarcinomas [3]. Approximately 80% of TCCs are low-grade tumors that are papillary, non-invasive and usually superficial, with stages Ta and Tis; the remaining 20% are high-grade papillary or non-papillary tumors that are often invasive or metastatic, with stages T1–T4. The five-year survival rate for TCC patients is 50%. The involvement of the bladder muscular wall signifies a worse prognosis and requires aggressive medical intervention such as radical cystectomy [4,5].

Occupational exposures in the textile and tire industries were the first factors implicated in the induction of bladder cancer. Currently, the prolonged use of phenacetin analgesics, exposure to cyclophosphamide, and smoking are the main risk factors associated with the etiology of transitional cell carcinoma [6]. Although men are 3-4 times more likely to develop bladder cancer, women present more often with advanced disease and have a lower probability of survival [7]. According to Shariat et al.[8], age is also considered a risk factor for urothelial carcinoma because the incidence of this cancer increases progressively with age; the incidence is higher after 60 years and peaks at 70 years, when the risk is 2% to 4% in men and 0.5% to 1% in women [9].

Clinically, the main problem associated with urothelial tumors is their highly unpredictable potential to progress to muscle-invasive disease, become multifocal and recur [5,10]. The recurrences might be *de novo* lesions that are different from recidivates, which occur because of incomplete resection of the primary tumor. After resection and/or treatment of a primary tumor, *de novo* TCC occurs in 50% to 70% of patients over a period of 4–5 years of follow-up. In fact, it has been suggested that patients undergoing surgical procedures are at a high risk for developing new neoplasia and are also susceptible to recurrences, possibly because of the presence of urothelial genetic instabilities [11–13].

Two hypotheses have been proposed to explain the association between urothelial carcinogenesis, multifocality and recurrence. The first hypothesis suggests a monoclonal origin of the lesions. In other words, multifocal or recurrent tumors originate from a single transformed cell that proliferates and colonizes other parts of the bladder through intraepithelial migration or transportation by urine. The second hypothesis proposes a polyclonal origin, suggesting that urine carcinogens that are in contact with multiple sites lead to the development of independent multifocal tumors [14,15]. The understanding of the clonality of multifocal bladder tumors is important to establish therapeutic strategies because new therapies often target specific molecules in these tumors [10].

2. DNA mutation and bladder carcinogenesis

Tumors are made up of billions of cells that originate from an initial cell that eluded apoptosis, accumulated genetic alterations and multiplied clonally [16]. It is expected that both external and internal factors contribute to these genetic mutations. External factors include lifestyle, such as excessive alcohol consumption, an unhealthy diet, exposure to excessive sunlight and chemical carcinogens, lack of exercise and smoking [17]. Internal factors include gene mutations, changes in the hormonal and immune systems, and metabolic abnormalities. During cell division, spontaneous genetic errors occur at an estimated frequency of approximately 10^{-5} to 10^{-6} [18]. Therefore, the blockade of apoptosis can favor the accumulation of mutated cells, a critical event in cancer pathogenesis [19].

Carcinogenesis is a multistep process that involves initiation, promotion and progression. Initiation is characterized by the formation of a preneoplastic cell resulting from an irreversible genotoxic event (gene mutation) caused by chemical, physical or biological carcinogens. This mutation usually occurs in genes that control the cell cycle, cell differentiation, apoptosis and DNA repair, leading to the survival of cells with genetic alterations [20]. The promotion stage involves the selective clonal expansion of the initiated cell through an increase in cell growth or a decrease in apoptosis, leading to an accumulation of mutations and an increase in the level of genetic instability (genetic and epigenetic changes) [20]. The third step, progression, involves genetic events such as changes in ploidy and chromosome integrity and results in a change from the preneoplastic state to the neoplastic state, producing cells with a high degree of anaplasia, an imbalance between cell proliferation and apoptosis and self-sufficiency (e.g., growth and multiplication independent of stimuli - Figure 1) [20,21].

Figure 1. Multistep process of carcinogenesis

Urinary bladder carcinogenesis also occurs through multiple stages that are characterized by genetic changes that reflect the malignant transformation of an initiated normal cell [22]. These changes can occur in oncogenes/protooncogenes, tumor suppressor gene, regions of microsatellites, and cell cycle regulatory genes [23], which can trigger a framework of genetic instability characterized by a significant increase in the mutation rate (an early event in carcinogenesis). Genetic instability can be divided into two types: the first type comprises the insertions/deletions (basic single nucleotide changes) that result in read errors and are often observed in microsatellite regions (microsatellite instability), and the second type comprises the loss or gain of whole chromosomes or chromosome fragments (chromosomal changes), resulting in the loss or amplification of regions of DNA that contain genes crucial for neoplastic development [24].

Several studies have shown that many genetic and molecular alterations are involved in the initiation and progression stages of TCC, although the mechanisms responsible for the malignant phenotype are not completely understood. It is known that the accumulation of genetic changes, and not just a single mutation, determines the clinical behavior of TCC [25]. In fact, several studies have demonstrated the existence of numerous chromosomal changes in neoplastic and non-neoplastic urothelial cells from patients with a history of bladder cancer. The most frequent changes are polysomy of chromosomes 3, 7 and 17 and monosomy of chromosome 9 [26-30]. Furthermore, some authors have observed that 100% of patients with chromosome 17 loss exhibit recurrence [31]. Genetic analyses have also shown that the oncogenes *RAS* (related to recurrence), *erb-B2* (related to cell survival) and *EGF/EGFR* (related to recurrence and tumor progression) are the most important prognostic markers for bladder cancer [32]. Microsatellite alterations on chromosome 9 are indicative of genomic instability [33], but chromosome 9q segment loss (in low-grade papillary TCC), *FGFR3* mutations (low grade non-invasive tumors with low potential of progression) and the loss of *TP53* function (associated with muscle-invasive disease and metastatic potential) have also been described

[34,35]. Additionally, some authors have reported that *SOCS-1*, *STAT-1*, *BCL-2*, *DAPK*, and *E-cadherin* gene methylation are linked to tumor recurrence [36].

The *TP53* tumor suppressor gene has an important role in the cellular responses to various stress agents, including DNA damage [37,38]. After DNA damage occurs, *TP53* induces the transient or permanent blockage of cell proliferation or activates cell death signaling pathways [39]. However, it has been shown that some mutations in human tumors abolish or attenuate the binding of p53 protein to its consensus DNA sequence, abolishing the transcriptional activation of *TP53* target genes and resulting in the partial or complete loss of p53 function [40]. In fact, some studies have demonstrated that bladder tumor cells are grouped based on their molecular alterations in the *TP53* and *RB* signaling pathways [41]. Several mutations were found to confer new functions to mutant p53 that are independent of the wild-type p53 [42]. These findings have several implications, including a possible heterogeneous clinical phenotype depending on whether p53 itself is mutated and the site of the mutations or whether the p53 function is indirectly modified [43]. It has been demonstrated that genes related to cellular communication, cell cycle, cell division, cell death, cellular component organization, cell adhesion, and cell proliferation pathways, among others, are closely associated with the tumor grade. Although gene networks vary according to the tumor grade, *TP53* and several other genes have been frequently shown to be associated with the malignant phenotype of bladder tumors [44]. Independent of the *TP53* status, differences have been reported in several signaling pathways, such as the AMP kinase, JAK/STAT3, and MAP kinase (p38 MAPK, ERK, JNK) pathways. The downregulation of the *adipoR1* (involved in the AMP kinase pathway), *ABCA7* (involved in the ERK phosphorylation pathway), *DUSP22* (involved in the ERK and MAPK pathways), and *AKAP7* (involved in second messenger-mediated signaling events) genes was observed in cells with different tumor grades. Similarly, genes related to transcription, replication and DNA synthesis are also differentially expressed independent of the *TP53* status [44]. Additionally, no relationship between tumor grade or *TP53* status and the expression of *ANLN* and *S100P* (genes used as progression biomarkers in some types of tumors) in TCC lines has been described [44].

In normal cells, the p53 level is regulated by the interaction of the proteins mdm2, cop1, jnk and pirh2, which promote p53 degradation (ubiquitin/proteasome pathway) (Figure 2). After exposure to genotoxic or non-genotoxic stressors, the level of p53 is increased because the interaction with mdm2 and other regulators is inhibited. Then, several modulators (kinases, acetylases, etc) activate p53 transcriptional activity. The final result of p53 activation is either cell cycle arrest and DNA repair or apoptosis (Figure 3) [45].

Smoking is usually associated with the development of persistent clones of DNA-damaged cells in the urothelium and may partially explain the continuous occurrence of genetically aberrant cells in the mucosa. It is important to note that increased DNA damage has been detected in the transitional cells of smokers and ex-smokers who are free of neoplasia and have normal urinary bladder cell cytology [46]. Cytogenetic analyses have shown that bladder tumor recurrence is associated with high levels of DNA damage, which are still present in the normal-appearing urothelium of patients surgically treated for TCC [12]. Data suggest that part of this damage might occur through both clastogenic and aneugenic events, as de-

tected by the micronucleus test (Figure 4) in TCC patients (J.P. Castro Marcondes personal communication, July 18, 2012). The increased level of DNA damage in cytologically “normal” cells from patients with a history of TCC has been shown to be related to the tumor histological grade, regardless of the length of time or clinical course since resection, suggesting these cells may be new TCC precursors or subclones of a previous TCC. Based on these data, it has been suggested that the primary tumor represents only the most obvious component of the disease, and several foci of secondary “reseeded” or “relocated” anomalous urothelium exist or may appear when the primary neoplasm is diagnosed [12]. Therefore, the genetic follow-up of patients after surgery must be a routine because elevated levels of DNA damage could predict recurrence.

Figure 2. The *TP53* gene and the p53 protein. A) The *TP53* locus: chromosome 17 (17p13.1); B) the p53 protein (1 - acidic transactivation domain and mdm2 protein binding site (amino-terminus), 2 - proline-rich region and second transactivation domain, 3 - DNA binding domain, 4 - oligomerization domain and 5 - non-specific DNA binding domain that binds to damaged DNA (carboxy-terminus)) and regulators. Adapted from [45].

Cystoscopy and cytology are considered standard procedures for monitoring patients with a history of TCC and individuals with bladder cancer symptoms (hematuria, pollakiuria and dysuria). However, these exams have a very limited ability to detect microscopic lesions and are subjective because they depend on the cytopathologist’s experience; therefore, these tests have very low sensitivity for low-grade lesions [47]. It has been shown that only 61% of patients with biopsies positive for TCC had a similar diagnosis based on the cytological analysis [48]. On the other hand, some authors have reported 100% agreement between biopsies and cytogenetic analysis results using probes for the centromeres of chromosomes 3, 7 and 17 and the 9p21 locus. Thus, the use of techniques that increase the sensitivity and specificity of early TCC detection, both in patients undergoing bladder tumor resection and in patients considered at risk for TCC, must be taken into consideration. In this context, biomarkers linked to the behavior of a particular biological entity (e.g., chromosome damage) might be used to assess cancer risk in different tissues.

Figure 3. Upstream and downstream p53 activation pathways. Adapted from [45].

Figure 4. Exfoliated urothelial cell with a micronucleus (arrow). Giemsa stain (X 1000). Adapted from [49].

3. Bladder cancer and chemotherapy

It is import to know the disease stage to effectively plan the treatment for bladder cancer. Different types of treatments are available, including surgery, biologic therapy, radiotherapy, and chemotherapy. TCC has been efficiently treated with radiotherapy and combinations of different antineoplastic compounds. Intravesical Bacillus Calmette Guérin (BCG)

instillations have shown success as adjuvant treatment for patients with intermediate and high risk non-muscle-invasive bladder tumor [50]. BCG induces a massive influx of cytokines and inflammatory cells into the bladder wall and lumen [51]. Moreover, BCG therapy has been demonstrated to reduce the recurrence rate and the risk of progression to muscle invasive disease in patients with carcinoma in situ and superficial bladder tumors [52].

Combined chemotherapy protocols have been extensively studied with the goal of improving bladder cancer treatment and the overall survival rate [53]. The standard protocol includes the drugs methotrexate, vinblastine, doxorubicin and cisplatin (MVAC) [54], but gemcitabine has also been successfully introduced [55]. The primary effect induced by these drugs is DNA damage with consequent cell cycle arrest and apoptosis. However, tumor cells have different levels of sensitivity to therapeutic agents, which may affect treatment success. Moreover, the genetic background of each tumor/patient must be taken into account to ensure treatment efficacy. In the context of developing chemotherapy protocols, the characterization of genes associated with a tumor's sensitivity to antitumor agents plays a critical role in the selection of the optimal treatment [56].

In 2000, Von der Maase et al. [54] demonstrated that the gemcitabine/cisplatin regimen had an efficacy similar to that of the MVAC protocol but with superior safety and tolerability, thus providing a potential standard alternative to treat bladder cancer. Gemcitabine is a deoxycytidine analog, which is phosphorylated to yield an active dFdCTP metabolite (gemcitabine triphosphate) that is incorporated into DNA, causing DNA strand breaks and thereby eliciting a DNA damage response characterized by cell cycle arrest in the G1/S phase and replication blockage [57,58]. Gemcitabine can also be incorporated into RNA to inhibit RNA synthesis [59]. Because of its low molecular weight of 299 Da, (lower than the molecular weights of drugs commonly used in intravesical chemotherapy; e.g., mitomycin C and doxorubicin), gemcitabine is able to penetrate the bladder mucosa, which has beneficial effects on the treatment of invasive bladder cancers [60]. Cisplatin is one of the most potent antitumor agents, with the ability to induce DNA crosslinking and apoptosis [61,62]. A molecule of cisplatin consists of a central atom of platinum surrounded by two chlorine atoms and two ammonia groups. Cisplatin is activated by the reaction of water molecules with the chloride ions. This activated compound then reacts with DNA, RNA, proteins and phospholipid membranes [63]. Similar to other platinum compounds, cisplatin forms DNA adducts between adjacent guanines (65%) and between guanine and adenine (25%) and forms inter-strand crosslinks (10%) that interfere with DNA replication and repair, contributing to its antitumor efficacy [64,65].

The *TP53* status had been shown to play a pivotal role in the response to a large panel of anticancer drugs. Numerous studies have investigated the relationship between the tumor suppressor protein p53 and/or *TP53* gene mutations and the response to chemotherapy. Cote et al. [66] demonstrated that the presence of a normal functional *TP53* is associated with a good response to chemotherapy, and Hall et al. [67] suggested that the existence of *TP53* allelic variants indicates a complex role for the *TP53* pathway in human neoplasias. Therefore, differences among *TP53* responses may reflect the complex biology of this gene with respect to the regulation of apoptosis and cell proliferation. Because the *TP53* network

is linked to many other cellular pathways, it is possible that defects in some of these pathways might qualitatively or quantitatively interfere with p53 function. Moreover, p53 is only one component of a giant surveillance network modulated by many other elements, including negative (Mdm2, Mdmx, Pirh2 and COP1) [68] and positive (DERP6) [69] regulators of p53, other members of the p53 family and several other signaling pathways [70].

The *TP53* and p53 status have also been used as biological markers to predict the response to chemotherapy. However, p53 expression and BCG response have shown contradictory results in literature. While some authors have concluded that p53 expression is not suitable as a marker to predict BCG response [71,72], other have stated that p53 has potential to be used as an independent marker to distinguish BCG responders and BCG non-responders in terms of time to recurrence and progression and progression to muscle invasive disease [73,74]. Moreover, independent on *TP53* status, some investigators have reported that the BCG therapy induces cellular reactive oxygen species and lipid peroxidation in cancer cells, inducing DNA damage, which could lead to mutations that select for their survival [75]. Thus, the authors suggest that reducing either the number of instillations of BCG that patients receive or the dose of BCG may reduce the amount of ROS and DNA damage and could lead to reduced disease progression [75]. Other authors have conclude that BCG response depend on the combination of markers to provide important information for selecting patients for the appropriate treatment [76].

On the other hand, there are few data in the literature regarding the relationship between this biomarker and the response to gemcitabine or cisplatin [77-80]. With regard to cell cycle kinetics, gemcitabine or combined treatment with gemcitabine plus cisplatin induces G1 cell cycle arrest in TCC cell lines *in vitro* independent of the *TP53* status. Conversely, only the cell responses to cisplatin were dependent on the *TP53* status. Whereas the wild-type *TP53* cells stopped in S phase, the *TP53*-mutated cells accumulated in G2 phase [81]. Similar findings have been described regarding apoptosis: whereas cisplatin induces apoptosis in only wt-*TP53* cells, apoptosis occurs in cells treated with gemcitabine or gemcitabine plus cisplatin independent of the *TP53* status, although higher percentages are observed in the wt-*TP53* cells [81]. In wt-*TP53* cells, gemcitabine-induced cellular damage can stimulate p53 expression, resulting in p21 expression and cell cycle arrest, enabling DNA damage repair or inducing apoptosis mediated by the *BAX* gene. In cells with a mutated *TP53* phenotype, the expression of p53 and p21 cannot be induced, but *BAX* can still be expressed, resulting in apoptosis [82]. Regarding cytotoxicity, *TP53*-wt cells were more resistant to cisplatin and more sensitive to gemcitabine than mutated *TP53* cells [81]. Some authors have suggested that the effect of cisplatin on human cancer cells has characteristics of senescence rather than apoptosis [83]. According to these authors, cancer cells lacking *TP53* function can also be killed via a *TP53*-independent mechanism, similar to replicative senescence. However, combined treatment with cisplatin and gemcitabine was more effective in reducing cell survival than treatment with the two drugs individually, independent of the *TP53* status [81]. Interestingly, genetic networks determined by Bayesian interpolation and built from microarray data show that, *in vitro*, TCC cell lines do not establish positive or negative relationships between *TP53* and a group of genes but instead exhibit direct interactions between *TP53* and

many genes. Furthermore, different gene networks have been observed according to the tumor cell lines were obtained, confirming that other genes and pathways are involved in the chemotherapy response, independent of the TP53 status [44]. It is known that both gemcitabine and cisplatin act by inducing DNA structural damage and modulating gene expression. Some authors have demonstrated that gemcitabine has cytotoxic and genotoxic effects in murine bone marrow [84], and other authors have confirmed the genotoxic effect of antineoplastic drugs in circulating blood lymphocytes [85]. Several studies revealed that cisplatin is an effective clastogen and inducer of both sister chromatid exchange and micronuclei development [86,87]. Furthermore, several authors have demonstrated that cisplatin induces a noticeable mutagenic effect, increasing the frequency of micronuclei and the percentage of chromosome aberrations in rat bone-marrow cells [88]. Additionally, Brozovic et al. [89] reported that cisplatin induces strong genotoxicity in murine peripheral blood leucocytes and brain, liver and kidney cells. In bladder cancer cells, gemcitabine and cisplatin, alone or in combination, have been shown to cause significant DNA damage at different tumor development stages independent of the TP53 status (Figure 5). However, TP53-mutated TCC cells are more resistant to the genotoxic effects induced by the combined treatment with gemcitabine and cisplatin than wild-type cells are (E.A de Carmargo personal communication, June 27, 2012). Regarding the toxicogenomic and proteomics events, Nordentoft et al. [90] demonstrated that the relationship between the transcription factor TFAP2 α and cisplatin or gemcitabine sensitivity in bladder cancer cells is dependent on p53 because TFAP2 α silencing increased the proliferation of only the wild type TP53 bladder cells and reduced cisplatin- and gemcitabine-induced cell death. Additionally, Gazzaniga et al [91] reported that gemcitabine induces apoptosis in TP53-mutated cells, involving caspase-3, -8 and -9 activation but no changes in *Bcl-2*, *Bax*, *survivin* and *Bcl-X* expression. In fact, the gemcitabine-induced modulation of *Bax* expression has been observed only in a wild-type TP53 cell line (Da Silva et al., 2012, unpublished data, [92]). In contrast, following treatment with gemcitabine or cisplatin plus gemcitabine, there was an observable upregulation of the *GADD45A* and *CDKN1A* genes independent of the TP53 status in bladder cancer cell lines, thus providing possible links to apoptosis and cell cycle arrest (Da Silva et al., 2012, unpublished data). On the other hand, Cho et al [93] reported that *Bcl-2* upregulation in a TP53 mutated bladder cancer cell line contributes to the development of cisplatin resistance, and targeting this gene with an siRNA may therefore be a potential tool to reverse cisplatin resistance. Matsui et al [94] also reported that the expression of the galectin-7 gene could serve as a candidate predictive marker for chemosensitivity to cisplatin in wild-type TP53 cells.

In conclusion, while there is evidence implicating the role of TP53 in the regulation of DNA repair and apoptosis and as a molecular node, other target genes can also be modulated by antineoplastic compounds and influence the success of drug therapy. Regardless of tumor-associated TP53 mutations or the tumor grade, simultaneous treatment with cisplatin and gemcitabine is an effective protocol for transitional cell carcinomas. In this context, because high concentrations of cisplatin are toxic to humans, the use of low concentrations of cisplatin and gemcitabine in combination might be clinically relevant in reducing the secondary effects of chemotherapy [81].

Figure 5. Genotoxic damage induced by cisplatin and gemcitabine in transitional carcinoma cells, as depicted by the comet assay. (A) Untreated cells; (B) cells treated with cisplatin; (C) cells treated with gemcitabine. Ethidium bromide staining (X 400).

4. Actual scenario

Most cellular components exert their functions by interacting with other components located within the same cell, in different cells, or even in different organs. In humans, the complexity of the interaction networks (the human interactome) is impressive: there are approximately 25,000 protein-coding genes, approximately 1,000 metabolites and an indefinite number of distinct proteins and functional RNA molecules. Therefore, the number of cellular components capable of being regulatory interactome centers exceeds 100000 [95]. Moreover, the intra- and inter-cellular connectivity implies that the impact of genetic abnormality is not restricted to the activity of the gene product but can have effects on other genes and their products that might have no defect. Several authors have suggested that the disease phenotype is rarely a consequence of abnormalities in a single gene product but reflects various patho-biological processes that interact in a complex network [96]. Therefore, the effects of cell interconnection on disease progression can lead to the identification of genes and systems that offer better targets for drug development. Moreover, the potential use of microRNA in the future therapeutic interventions has also been discussed. For example, the effects of miR-100 on cell growth and clonogenic capacity in TCC cell lines emphasize a possible link between this miRNA and bladder carcinoma pathogenesis [97]. These new concepts may identify more accurate biomarkers for monitoring the functional integrity of networks and classifying diseases [96].

Changes in gene expression profiles may be immediate and more sensitive markers of drug toxicity than markers that are typically analyzed in toxicity tests (morphological changes, carcinogenicity and reproductive markers) [98]. Furthermore, some authors have shown that the implementation of proteomic platforms for the identification of novel targets of interest (membrane antigens, protein overexpression, etc.) is gaining widespread attention. The incorporation of biomarkers in clinical proteomics studies has also become important to define biologically effective therapeutic protocols for each patient and type of disease [99]. Thus, studies comparing gene and protein expression can confirm and emphasize the im-

portance of using different technologies to understand and characterize complex biological systems.

5. Final conclusion

In this chapter, we presented data that demonstrate that high levels of DNA damage in normal-appearing urothelium are associated with tumor recurrence in patients treated for bladder TCC. Furthermore, the identification of genes associated with the sensitivity of tumors to chemotherapeutic drugs may play an important role in selecting the most efficient treatment protocol. Therefore, biomarker identification is relevant not only for diagnostic accuracy and prognosis but also for cancer therapy.

Currently, the ability of genomics and proteomics techniques to identify biomarkers and increase our understanding of complex cellular networks has been demonstrated. Thus, high-throughput methodologies help characterize diseases and increase our understanding of tumor progression mechanisms and the chemotherapy results. It is known that the primary effects of antineoplastic drugs are linked to DNA damage, leading to molecular events that may result in cell cycle arrest and apoptosis, which are essential responses for the maintenance of genetic integrity and cell viability [100]. Furthermore, it is known that early detection and treatment result in better survival rates for patients without clinical symptoms during the early stages of carcinogenesis [101].

Abbreviations

BCG – Baccillus Calmette Guérin

TCC – Transitional cell carcinoma

MVAC - Methotrexate, Vinblastine, Doxorubicin and Cisplatin

Author details

Daisy Maria Favero Salvadori* and Glenda Nicioli da Silva

*Address all correspondence to: dfavero@fmb.unesp.br

UNESP – Universidade Estadual Paulista; Botucatu Medical School; Department Pathology, Botucatu, Brazil

References

- [1] Frau DV, Usai P, Dettori T, Caria P, De Lisa A, Vanni R. Fluorescence in situ hybridization patterns in newly diagnosed superficial bladder lesions and corresponding bladder washings. *Cancer Genetics and Cytogenetics* 2006;169(1) 21–26.
- [2] Shelley MD, Mason MD, Kynaston H. Intravesical therapy for superficial bladder cancer: a systematic review of randomised trials and meta-analyses. *Cancer Treatment Reviews* 2010;36(6) 195–205.
- [3] Cordon-Cardo C. Molecular alterations associated with bladder cancer initiation and progression. *Scandinavian Journal of Urology and Nephrology* 2008;218 154–165.
- [4] Kaufman DS. Challenges in the treatment of bladder cancer. *Annual Oncology* 2006;17(5) 106–112.
- [5] Castillo-Martin M, Domingo-Domenech J, Karni-Schmidt O, Matos T, Cordon-Cardo C. Molecular pathways of urothelial development and bladder tumorigenesis. *Urology Oncology* 2010;28(4) 401–408.
- [6] Wallerand H, Bakkar AA, Diez de Medina SG, Pairon JC, Yang YC, Vordos D. Mutations in TP53, but not FGFR3, in urothelial cell carcinoma of the bladder are influenced by smoking: contribution of exogenous versus endogenous carcinogens. *Carcinogenesis* 2005; 26(1) 177–184.
- [7] Fajkovic H, Halpern JA, Cha EK, Bahadori A, Chromecki TF, Karakiewicz PI, Breinl E, Merseburger AS, Shariat SF. Impact of gender on bladder cancer incidence, staging, and prognosis. *World Journal of Urology* 2011;29(4) 457–463.
- [8] Shariat SF, Milowsky S, Droller MJ, M.D. Bladder cancer in the elderly. *Urology Oncology* 2009;27 653–667.
- [9] Kirkali Z, Chan T, Manoharan M, Algaba F, Bush C, Cheng L, et al. Bladder Cancer: Epidemiology, staging and grading, and diagnosis. *Urology* 2005;66(6):4–34.
- [10] Denzinger S, Mohren K, Knuechel R, Wild PJ, Burger M, Wieland WF, Hartmann A, Stoehr R. Improved clonality analysis of multifocal bladder tumors by combination of histopathologic organ mapping, loss of heterozygosity, fluorescence in situ hybridization, and p53 analyses. *Human pathology* 2006;37(2) 143–157.
- [11] Nilsson S, Ragnhammar P, Nygren P, Glimelius B. A Systematic Overview of chemotherapy effects in urothelial bladder cancer. *Acta oncologic* 2001;40(2-3) 371–390.
- [12] Gontijo AM, Marcondes JPC, Elias FN, de Oliveira MLCS, Lima ROA, Salvadori DMF, Camargo JLV. DNA Damage in Cytologically Normal Urothelial Cells of Patients With a History of Urothelial Cell Carcinoma. *Environmental and Molecular Mutagenesis* 2002;40(3) 190–199.

- [13] Latini DM, Lerner SP, Wade SW, Lee DW, Quale DZ. Bladder cancer detection, treatment and outcomes: opportunities and challenges. *Urology* 2010;75(2) 334–339.
- [14] Hafner C, Knuechel R, Stoehr R, Hartmann A. Clonality of multifocal urothelial carcinomas: 10 years of molecular genetic studies. *International Journal of Cancer* 2002;101(1) 1-6.
- [15] Paiss T, Wöhr G, Hautmann RE, Mattfeldt T, Müller M, Haeussler J, Vogel W. Some tumors of the bladder are polyclonal in origin. *Journal of Urology* 2002;167(2 Pt 1) 718-723.
- [16] Trosko JE. Commentary: is the concept of “tumor promotion” a useful paradigm? *Molecular Carcinogenesis* 2001;30(3) 131– 137.
- [17] Sankpal UT, Pius H, Khan M, Shukoor MI, Maliakal P, Lee CM, Abdelrahim M, Connelly SF, & Riyaz Basha. Environmental factors in causing human cancers: emphasis on tumorigenesis. *Tumor Biology* 2012. [Epub ahead of print]
- [18] Cohen SM, Lawson TA. Rodent bladder tumors do not always predict for humans. *Cancer Letters* 1995;93(1) 9–16.
- [19] Qu W, Bortner CD, Sakurai T, Hobson MJ, Waalkes MP. Acquisition of apoptotic resistance in arsenic-induced malignant transformation: role of the JNK signal transduction pathway. *Carcinogenesis* 2002;23(1) 151–159.
- [20] Vicent TL, Gatenby RA. An evolutionary model for initiation, promotion, and progression in carcinogenesis. *International Journal of Oncology* 2008;32(4) 729-737.
- [21] Pitot, HC. Adventures in hepatocarcinogenesis. *Annual Reviews of Pathology* 2007;2 1-29.
- [22] Philips JL, Richardson IC. Aneuploidy in bladder cancers: the utility of fluorescent in situ hybridization in clinical practice. *BJU International* 2006, 98(1) 33-37.
- [23] Habuchi T, Marberger M, Droller MJ, Hemstreet III GP, Grossman HB, Schalken JA, et al. Prognostic markers for bladder cancer: international consensus panel on bladder tumor markers. *Journal of Urology* 2005;66(6A) 64-74.
- [24] Catto JWF, Meuth M, Hamdy FC. Genetic instability and transitional cell carcinoma of the bladder. *BJU International* 2004;93(1) 19-24.
- [25] Kim IY, Kim SJ. Role of bone morphogenetic proteins in transitional cell carcinoma cells. *Cancer Letters* 2006;241(1) 118-123.
- [26] Kruger S, Mess F, Bohle A, Feller AC. Numerical aberrations of chromosome 17 and the 9p21 locus are independent predictors of tumor recurrence in non-invasive transitional cell carcinoma of the urinary bladder. *International Journal of Oncology* 2003;23(1): 41-48.
- [27] Obermann EC, Meyer S, Hellge D, Zaak D, Filbeck T, Stoehr R, Hofstaedter F, Hartmann A, Knuechel R. Fluorescence in situ hybridization detects frequent chromo-

some 9 deletions and aneuploidy in histologically normal urothelium of bladder cancer patients. *Oncology Reports* 2004;11(4): 745-751.

- [28] Latif Z, Watters AD, Dunn I, Grigor K, Underwood MA, Bartlett JM. HER2/neu gene amplification and protein overexpression in G3 pT2 transitional cell carcinoma of the bladder: a role for anti-HER2 therapy? *European Journal of Cancer* 2004;4(1) 56-63.
- [29] Degtar P, Neulander E, Zirkin H, Yusim I, Douvdevani A, Mermershtain W et al. Fluorescence in situ hybridization performed on exfoliated urothelial cells in patients with transitional cell carcinoma of the bladder. *Urology* 2004;63(2) 398-401.
- [30] Pycha A, Lodde M, Comploj E, Negri G, Egarter-Vigl E, Vittadello F et al. Intermediate-risk urothelial carcinoma: na unresolved problem? *Urology* 2004;63(3) 472-475.
- [31] Ishiwata S, Takahashi S, Homma Y, Tanaka Y, Kameyama S, Hosaka Y, Kitamura T. Noninvasive detection and prediction of bladder cancer by fluorescence in situ hybridization analysis of exfoliated urothelial cells in voided urine. *Urology* 2001;57(4) 811-815.
- [32] Kausch I, Bohle A. Molecular aspects of bladder cancer III. Prognostic markers of bladder cancer. *European Urology* 2002;41(1) 15-29.
- [33] Turyn J, Matuszewski M, Schlichtholz B. Genomic instability analysis of urine sediment versus tumor tissue in transitional cell carcinoma of the urinary bladder. *Oncology Reports* 2006;15(1) 259-265.
- [34] Baithun Si, Naase M, Blanes A, Diaz-Cano Sj. Molecular and kinetic features of transitional cell carcinomas of the bladder: biological and clinical implications. *Virchows Archives* 2001;438(3) 289-297.
- [35] Cheng L, Zhang S, MacLennan GT, Williamson SR, Lopez-Beltrn, Montironi R, FRCPath, IFCAP. Bladder cancer: translating molecular genetic insights into clinical practice. *Human Pathology* 2011;42(4) 455-481.
- [36] Friedrich MG, Chandrasoma S, Siegmund KD, Cheng JC, Toma MI. Prognostic relevance of methylation markers in patients with non-muscle invasive bladder carcinoma. *European Journal of Cancer* 2005;41(17) 1009-1015.
- [37] Kosmider B, Osiecka R, Zyner E, Ochocki J. Comparison between the genotoxicity of cis-Pt(II) complex of 3-aminoflavone and cis-DDP in lymphocytes evaluated by the comet assay. *Drug and Chemical Toxicology* 2005;28(2) 231-244.
- [38] Basu A, Krishnamurthy S. Cellular Responses to Cisplatin-Induced DNA Damage. *Journal of Nucleic Acids* 2010, pii: 201367.
- [39] Kim HG, Lee S, Kim DY, Ryu SY, Joo JK, Kim JC, Lee KH, Lee JH. Aberrant methylation of DNA Mismatch repair genes in elderly patients with sporadic gastric carcinoma: a comparison with younger patients. *Journal of Surgical Oncology* 2010;101(1) 28-35.

- [40] Kato S. Understanding the function structure and function mutation relationships of p53 tumor suppressor protein by high resolution missense mutation analysis. *Proceedings of the National Academy of Sciences USA* 2003;100(14) 8424–8429.
- [41] Sanchez-Carbayo M, Socci ND, Charytonowicz E, Lu M, Prystowsky M, Childs G, Cordon-Cardo C. Molecular profiling of bladder cancer using cDNA microarrays: defining histogenesis and biological phenotypes. *Cancer Research* 2002;62(23) 6973–6980.
- [42] Brosh R, Rotter V. When mutations gain new powers: news from the mutant p53 field. *Nature Review Cancer* 2009;9(10) 701–713.
- [43] Prives C, Manfredi JJ. The continuing saga of p53—More sleepless nights ahead. *Molecular Cell* 2005;19(6) 719–721.
- [44] da Silva GN, Evangelista AF, Magalhães DA, Macedo C, Búfalo MC, Sakamoto-Hojo ET, Passos GA, Salvadori DM. Expression of genes related to apoptosis, cell cycle and signaling pathways are independent of TP53 status in urinary bladder cancer cells. *Molecular Biology Reports* 2011;38(6) 4159–4170.
- [45] The TP53 website: <http://p53.free.fr/index.html> (accessed 20 July 2012).
- [46] Gontijo AM, Elias FN, Salvadori DM, de Oliveira ML, Correa LA, Goldberg J, Trindade JC, de Camargo JL. Single-Cell Gel (Comet) Assay Detects Primary DNA Damage in Nonneoplastic Urothelial Cells of Smokers and Ex-smokers. *Cancer Epidemiology, Biomarkers & Prevention* 2001;10(9) 987–993.
- [47] Fracasso ME, Franceschetti P, Doriaa D, Talamini G, Bonetti F. DNA breaks as measured by the alkaline comet assay in exfoliated cells as compared to voided urine cytology in the diagnosis of bladder cancer: a study of 105 subjects. *Mutation Research* 2004;564(1) 57–64.
- [48] Daniely M, Rona R, Kaplan T, Olsfanger S, Elboim L, Zilberstein Y et al. Combined analysis of morphology and fluorescence in situ hybridization significantly increases accuracy of bladder cancer detection in voided urine samples. *Urology* 2005;66(6) 1354–1359.
- [49] Marcondes JPC. Cytogenetic damage in exfoliated urothelial cell from patients with history of bladder transitional cell carcinoma. PhD thesis. Universidade Estadual Paulista; 2007.
- [50] Babjuk M, Oosterlinck W, Sylvester R, Kaasinen E, Böhle A, Palou-Redorta J, European Association of Urology (EAU). EAU guidelines on nonmuscle-invasive urothelial carcinoma of the bladder. *European Urology* 2008;54(2) 303–314.
- [51] Kresowik TP, Griffith TS. Bacillus Calmette–Guerin immunotherapy for urothelial carcinoma of the bladder. *Immunotherapy* 2009;1(2) 281–288.
- [52] Sylvester RJ, van der Meijden AP, Witjes JA, Kurth K. Bacillus Calmette–Guerin versus chemotherapy for the intravesical treatment of patients with carcinoma in situ of

- the bladder: a meta-analysis of the published results of randomized clinical trials. *Journal of Urology* 2005;174(1) 86–91.
- [53] Gallagher DJ, Milowsky MI, Bajorin DF. Advanced Bladder Cancer: Status of First-line Chemotherapy and the Search for Active Agents in the Second-line Setting. *Cancer* 2008; 113(6) 1284–1293.
- [54] von der Maase H, Hansen SW, Roberts JT, Dogliotti L, Oliver T, Moore MJ, Bodrogi I, Albers P, Knuth A, Lippert CM, Kerbrat P, Sanchez Rovira P, Wersall P, Cleall SP, Roychowdhury DF, Tomlin I, Visseren-Grul CM, Conte PF. Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin and cisplatin in advanced or metastatic bladder cancer: Results of a large, randomized, multinational, multicenter, phase III study. *Journal of Clinical Oncology* 2000;18(17) 3068-3077.
- [55] Bellmut J, Albiol S, Ramirez de Olano A, Pujadas J, Maroto P. On behalf the Spanish Oncology Genitourinary Group (SOGUG). Gemcitabine in the treatment of advanced transitional cell carcinoma of the urothelium. *Annual Oncology* 2006;17 113–117.
- [56] Fujita H, Ohuchida K, Mizumoto K, Itaba S, Ito T, Nakata K, Yu J, Kayashima T, Souzaki R, Tajiri T, Manabe T, Ohtsuka T, Tanaka M. Gene expression levels as predictive markers of outcome in pancreatic cancer after gemcitabine-based adjuvant chemotherapy. *Neoplasia* 2010;12(10) 807-817.
- [57] Galmarini CM, Clarke ML, Falette N, Puisieux A, Mackey JR, Dumontet C. Expression of a non-functional p53 affects the sensitivity of cancer cells to gemcitabine. *International Journal of Cancer* 2002;97(4) 439-445.
- [58] Toschi L, Finocchiaro G, Gioia V. Role of gemcitabine in cancer therapy. *Future Oncology* 2005;1 7-17.
- [59] Ruiz Van Haperen VWT, Veerman G, Vermoken JB, Peters GJ. 2',2'-Difluoro-deoxycytidine (gemcitabine) incorporation into RNA and DNA from tumor cell lines. *Biochemical Pharmacology* 1993;46(4) 762-766.
- [60] Gontero P, Freja B. Actual experience and future development of gemcitabine in superficial bladder cancer. *Annual Oncology* 2006;17(5) 123-128.
- [61] Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. *Nature Reviews* 2005;4(4) 307-319.
- [62] Shimabukuro F, Neto CF, Sanches Jr J.A, Gattás GJF. DNA damage and repair in leukocytes of melanoma patients exposed in vitro to cisplatin. *Melanoma Research* 2011;21(2) 99-105.
- [63] Cho JM, Manandhar S, Lee HR, Park HM, Kwak MK. Role of the Nrf2- antioxidant system in cytotoxicity mediated by anticancer cisplatin: Implication to cancer cell resistance. *Cancer Letters* 2008;260(1-2) 96–108.

- [64] Rabik CA, Dolan ME. Molecular mechanisms of resistance and toxicity associated with platinating agents. *Cancer Treatment Review* 2007;33(1) 9–23.
- [65] Stadler WM, Lerner SP, Groshen S, Stein JP, Shi SR, Raghavan D, Esrig D, Steinberg G, Wood D, Klotz L, Hall C, Skinner DG, Cote RJ. Phase III study of molecularly targeted adjuvant therapy in locally advanced urothelial cancer of the bladder based on p53 status. *Journal of Clinical Oncology* 2011;29(25) 3443-3449.
- [66] Cote RJ, Esrig D, Groshen S, Jones PA, Skinner DG. P53 and treatment of bladder cancer. *Nature* 1997;385 124-125.
- [67] Hall PA, McCluggage WG. Assessing p53 in clinical contexts: unlearned lessons and new perspectives. *Journal of Pathology* 2006;208(1) 1-6.
- [68] Wang L, He G, Zhang P, Wang X, Jiang M, Yu L. Interplay between MDM2, MDMX, Pirh2 and COP1: the negative regulators of p53. *Molecular Biology Reports* 2010;38(1) 229-236.
- [69] Yuan J, Tang W, Luo K, Chen X, Gu X, Wan B, Yu. Cloning and characterization of the human gene DERP6, which activates transcriptional activities of p53. *Molecular Biology Reports* 2006;33(3) 151–158.
- [70] Soussi T, Wiman KG. Shaping genetic alterations in human cancer: the p53 mutation paradigm. *Cancer Cell* 2007;12(4) 303–312.
- [71] Esuvaranathan K, Chiong E, Thamboo TP, Chan YH, Kamaraj R, Mahendran R, Teh M. Predictive value of p53 and pRb expression in superficial bladder cancer patients treated with BCG and interferon-alpha. *Cancer* 2007;109(6) 1097–1105.
- [72] Peyromaure M, Weibing S, Sebe P, Verpillat P, Toubanc M, Dauge MC, Boccon-Gibod L, Ravery V. Prognostic value of p53 overexpression in T1G3 bladder tumors treated with bacillus Calmette-Guérin therapy. *Urology* 2002;59(3) 409–413.
- [73] Saint F, Le Frere Belda MA, Quintela R, Hoznek A, Patard JJ, Bellot J, Popov Z, Zafrani ES, Abbou CC, Chopin DK, de Medina SG. Pretreatment p53 nuclear overexpression as a prognostic marker in superficial bladder cancer treated with bacillus Calmette-Guérin (BCG). *European Urology* 2004;45(4) 475–482.
- [74] Palou J, Algaba F, Vera I, Rodriguez O, Villavicencio H, Sanchez-Carbayo M. Protein expression patterns of ezrin are predictors of progression in T1G3 bladder tumours treated with nonmaintenance bacillus Calmette-Guérin. *European Urology* 2009;56(5) 829–36.
- [75] Rahmat JN, Esuvaranathan K, Mahendran R. Bacillus Calmette-Guérin induces cellular reactive oxygen species and lipid peroxidation in cancer cells. *Urology* 2012; 79(6) 1411.e15-20.
- [76] Zuiverloon TC, Nieuweboer AJ, Vékony H, Kirkels WJ, Bangma CH, Zwarthoff EC. Markers predicting response to bacillus Calmette-Guérin immunotherapy in high-

- risk bladder cancer patients: a systematic review. *European Urology* 2012;61(1) 128-145.
- [77] Kielb SJ, Nikhil LS, Rubin MA, Sanda MG. Functional p53 mutation as a molecular determinant of paclitaxel and gemcitabine susceptibility in human bladder cancer. *Journal of Urology* 2001;166(2) 482–487.
- [78] Fechner G, Perabo FGE, Schmidt DH, Haase L, Ludwig E, Schueller H, Blatter J, Muller C, Albers P. Preliminary evaluation of a radiosensitizing effect of gemcitabine in p53 mutant and p53 wild type bladder cancer cells. *Urology* 2003;61(2) 468–473.
- [79] Cory AH, Cory JG. Gemcitabine-induced apoptosis in a drug-resistant mouse leukemia L1210 cell line that does not express p53. *Advances in Enzyme Regulation* 2004;44 11–25.
- [80] Yip HT, Chopra R, Chkrabarti R, Veena MS, Ramamurthy B, Srivatsan ES, Wang MB. Cisplatin-induced growth arrest of head and neck cancer cells correlates with increased expression of p16 and p53. *Archives of Otolaryngology – Head & Neck Surgery* 2006;132 (3) 317–26.
- [81] da Silva GN, de Castro Marcondes JP, de Camargo EA, da Silva Passos Júnior GA, Sakamoto-Hojo ET, Salvadori DM. Cell cycle arrest and apoptosis in TP53 subtypes of bladder carcinoma cell lines treated with cisplatin and gemcitabine. *Experimental Biology and Medicine (Maywood)* 2010;235(7) 814-824.
- [82] Bergman AM, Pinedo HM, Peters GJ. Determinants of resistance to 2',2'- difluorodeoxycytidine (gemcitabine). *Drug Resistance Updates* 2002;5(1) 19-33.
- [83] Wang X, Wong SC, Pan J, Tsao SW, Fung KH, Kwong DL, Sham JS, Nicholls JM. Evidence of cisplatin-induced senescent-like growth arrest in nasopharyngeal carcinoma cells. *Cancer Research* 1998;58(22) 5019–5022.
- [84] Aydemir N, Bilaloğlu R. Genotoxicity of two anticancer drugs, gemcitabine and topotecan in mouse bone marrow in vivo. *Mutation Research* 2003;537(1) 43-51.
- [85] Cavallo D, Ursini CL, Perniconi B, Francesco AD, Giglio M, Rubino FM, Marinaccio A, Iavicoli S. Evaluation of genotoxic effects induced by exposure to antineoplastic drugs in lymphocytes and exfoliated buccal cells of oncology nurses and pharmacy employees. *Mutation Research* 2005;10:587(1-2) 45-51.
- [86] Choudhury RC, Jagdale MB, Misra S. Cytogenetic toxicity of cisplatin in bone marrow cells of Swiss mice. *Journal of Chemotherapy* 2000;12(2) 173-182.
- [87] Kosmider B, Wyszynska K, Janik-Spiechowicz E, Osiecka R, Zyner E, Ochocki J, Ciesielska E, Wasowicz W. Evaluation of the genotoxicity of cis-bis(3-aminoflavone)dichloroplatinum(II) in comparison with cis-DDP. *Mutation Research* 2004; 14:558(1-2) 93-110.
- [88] Rjiba-Touati K, Ayed-Boussema I, Skhiri H, Belarbia A, Zellema D, Achour A, Bacha H. Induction of DNA fragmentation, chromosome aberrations and micronuclei by

cisplatin in rat bone-marrow cells: Protective effect of recombinant human erythropoietin. *Mutation Research*. 2012;747(2) 202-206.

- [89] Brozovic G, Orsolic N, Knezevic F, Horvat Knezevic A, Benkovic V, Sakic K, Borojevic N, Dikic D. The in vivo genotoxicity of cisplatin, isoflurane and halothane evaluated by alkaline comet assay in Swiss albino mice. *Journal of Applied Genetics* 2011;52(3) 355-361.
- [90] Nordentoft I, Dyrskjot L, Bødker JS, Wild PJ, Hartmann A, Bertz S, Lehmann J, Orntoft TF, Birkenkamp-Demtroder K. Increased expression of transcription factor TFAP2 α correlates with chemosensitivity in advanced bladder cancer. *BMC Cancer*. 2011;11 135. PubMed PMID: 21489314
- [91] Gazzaniga P, Silvestri I, Gradilone A, Scarpa S, Morrone S, Gandini O, Gianni W, Frati L, Aglianò AM. Gemcitabine-induced apoptosis in 5637 cell line: an in-vitro model for high-risk superficial bladder cancer. *Anticancer Drugs* 2007;18(2) 179-85.
- [92] Da Silva GN, Camargo EA, Salvadori DMF. Toxicogenomic activity of gemcitabine in two TP53-mutated bladder cancer cell lines: special focus on cell cycle-related genes. *Molecular Biology Reports* 2012; DOI 10.1007/s11033-012-1916-1.
- [93] Cho HJ, Kim JK, Kim KD, Yoon HK, Cho MY, Park YP, Jeon JH, Lee ES, Byun SS, Lim HM, Song EY, Lim JS, Yoon DY, Lee HG, Choe YK. Upregulation of Bcl-2 is associated with cisplatin-resistance via inhibition of Bax translocation in human bladder cancer cells. *Cancer Letters* 2006;237(1) 56-66.
- [94] Matsui Y, Ueda S, Watanabe J, Kuwabara I, Ogawa O, Nishiyama H. Sensitizing effect of galectin-7 in urothelial cancer to cisplatin through the accumulation of intracellular reactive oxygen species. *Cancer Research* 2007;67(3) 1212-1220.
- [95] Zhao Y, Jensen ON. Modification-specific proteomics: strategies for characterization of post-translational modifications using enrichments techniques. *Proteomics* 2009;9(20) 4632-4641.
- [96] Barabasi A-L, Gulbahce N, Loscalo J. Network medicine: a network-based approach to human disease. *Nature Reviews* 2011;12(1) 56-68.
- [97] Oliveira JC, Brassesco MS, Morales AG, Pezuk JA, Fedatto PF, da Silva GN, Srideli CA, Tone LG. MicroRNA-100 Acts as a Tumor Suppressor in Human Bladder Carcinoma 5637 Cells. *Asian Pacific Journal of Cancer Prevention* 2011;12(11) 3001-3004.
- [98] Lee K-M, Kim J-H, Kang D. Design issues in toxicogenomics using DNA microarray experiment. *Toxicology and Applied Pharmacology* 2005;207(2) 200-208.
- [99] Lee J-M, Han JJ, Altwerger G, Kohn EC. Proteomics and biomarkers in clinical trials for drug development. *Journal of Proteomics* 2011;74(12) 2632-2641.

- [100] Tannock IF, Lee C. Evidence against apoptosis as a major mechanism for reproductive cell death following treatment of cell lines with anti-cancer drugs. *British Journal of Cancer* 2001;84(1) 100–105.
- [101] Spitz MR, Bondy ML. The evolving discipline of molecular epidemiology of cancer. *Carcinogenesis* 2010;31(1) 127–134.

IntechOpen

IntechOpen