JGG 2018;66:260-264

Review

# Age, mitochondria and bladder cancer

A. Cormio[1], C. Musicco[2], V. Pesce[1], R. Villani[3], A. Antonelli[4], E. Barret[5]

[1] Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Italy; [2] CNR-Institute of Biomembranes and Bioenergetics, Bari, Italy; [3] Department of Clinical and Experimental Medicine, University of Foggia, Italy; [4] Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia, Urology Unit, ASST-Ospedali Civili, Brescia, Italy; [5] Department of Urology, Institut Montsouris, Paris, France

Bladder cancer (BC) is a major cause of mortality worldwide. Risk factors for BC development are male gender, age and exposure to carcinogens and especially cigarette smoking. Since it is not clearly understood why aging is an important BC risk factor, in this review we try to find common features between BC and aging. Evidence suggest that oxidative stress and mitochondrial dysfunction may link aging to BC, thus opening new perspectives in terms of preventive measures and novel potential therapeutic targets.

**Key words:** Aging, Bladder cancer, Oxidative stress, Mitochondrial dysfunction

## INTRODUCTION

Bladder cancer (BC) is the ninth most common cancer worldwide. The most common risk factors for BC are smoking, male gender, age and exposure to environmental or occupational carcinogens such as arsenic, chromium, nickel and cadmium [1].

Carcinogens may trigger BC by inducing excessive reactive oxygen species (ROS) production and oxidative stress. High concentrations of ROS are pathogenic and can cause severe damage to cell and organelle membranes, DNA, and proteins, thus leading to cancer [2]. There is strong evidence linking oxidative stress and BC. Serum levels of vitamins C and E, whole blood levels of antioxidant enzymes like Superoxide dismutase and Glutathione peroxidase, and serum antioxidants were found to be significantly lower in patients than in controls, whereas serum malondialdehyde levels were found to be significantly higher, indicating presence of oxidative stress in BC patients [3]. Moreover, with advancing stage of BC, the levels of oxidative stress increase while the levels of antioxidant molecules decrease suggesting that they may be important factors in tumor development and growth [4]. The oxidative stress is one of the key hallmarks also of the aging process and is linked to the development of numerous age-related diseases including cancer. Therefore, ROS, oxidative damage, aging, and aging-dependent diseases like cancer seem to be connected.

Mitochondria are the center of the oxidative metabolism and the principal site of ROS production. According to the "Mitochondrial free radical theory of aging", aging is associated with progressive mitochondrial dysfunction [5]. This process is due to accumulation of mitochondrial DNA (mtDNA) mutations and increased ROS production leading to oxidative damage to cellular macromolecules, decline in mitochondrial quality control, reduced activity of metabolic enzymes, as well as changes in mitochondrial morphology, functionality and finally to reduced respiratory chain activity and adenosine triphosphate (ATP) generation [6].

The risk of BC increases with age, with age-specific curves increasing steeply after the age of 50 yr [1]. Since the population is aging, BC will become an even bigger public health challenge in the future. This review therefore aims to find a possible link between aging and BC that can provide novel opportunities for prevention and treatment of this disease.

Received: October 29, 2018 - Accepted: November 05, 2018

Correspondence: Luigi Cormio, Urology and Renal Transplantation Unit, Department of Medical and Surgical Sciences, University of Foggia, viale Luigi Pinto 251, 71122 Foggia, Italy. Tel.+39 0881 732111. Fax +39 0881 736056. E-mail: luigi.cormio@unifg.it

## MITOCHONDRIA

Mitochondria are essential organelles in all eukaryotic cells. They are the powerhouse that provides ATP for a multitude of cellular processes by the oxidative phosphorylation system. They are the hub of metabolic pathways, primary sources of ROS, regulators of apoptosis as well as signal transduction regulators, and buffers of intracellular calcium [7]. Mitochondria contain mitochondrial DNA (mtDNA), a small DNA of approximately 16569 bp, which codes for 2 rRNAs (12S and 16S), 22 tRNAs, and 13 proteins subunits of four of the five complexes of the respiratory chain [8]. MtDNA is more susceptible to ROS-induced mutations (point mutations or deletions) than nuclear DNA since it is located close to mitochondrial respiratory chain, the major source of ROS in the cell. Point mutations and deletions are the two most frequent types of mutations that arise in mtDNA genome mainly due to spontaneous errors during mtDNA replication or damage repair.
Not all mtDNA mutations, however, are deleterious to cells; some may result in dangerous events, others may be simple neutral polymorphisms with no important functional consequences. Moreover, it is important to know the percentage of mutant mtDNA molecules (threshold) that can lead to a dysfunction of the mitochondrial respiratory apparatus. A pathogenic mutation would need to rise up from 60 to over 95% of level to have a functional impact on the respiratory chain [9].
Apart from alterations of mtDNA (deletions, point mutations, and copy number), mitochondrial dysfunctions may also involve altered expression or damage of mitochondrial proteins and enzymes coded by nuclear DNA. To ensure maximal mitochondrial function, the mitochondrial quality control systems is active to protect mitochondria from ROS damage at the protein, DNA, and organelle level. At the protein level, mitochondria are protected by antioxidant systems, DNA repair, protein folding and degradation. At organelle level, damage activates mitochondrial biogenesis (*de novo* synthesis of mitochondria), mitochondrial dynamics (fusion and fission of mitochondria) and mitochondrial autophagy, also known as mitophagy [10 11]. Mitochondrial number and shape depend on mitochondrial biogenesis and dynamics. Mitochondria continuously join by the process of fusion and divide by the process of fission. Fusion is mediated by mitofusin-1 (Mfn1) and mitofusin-2 (Mfn2) in the outer mitochondrial membrane and by OPA1 in the inner membrane; Fission is mediated by dynamin related protein 1 (Drp1) and mitochondrial fission 1 protein (Fis1) [12]. The machinery regulating mitochondrial dynamics is highly integrated with mitophagy, with which it plays a role in mitochondrial quality control. Upon fission, mitochondria can be segregated into polarized and depolarized daughter mitochondria. While polarized daughter mitochondria can undergo fusion, depolarized mitochondria are targeted by mitophagic proteins to degradation [13 14]. Mitophagy promotes turnover of dysfunctional mitochondria that would otherwise hamper the cell homeostasis [15]. Important proteins in mitochondrial protein quality control systems are Lon protease and CLPP, two ATP-dependent protease located in mitochondrial matrix that contribute to the degradation of abnormal proteins as well as the maintenance of mitochondrial function [11 16].

### Mitochondrial alterations in aging

The mitochondrial free radical theory of aging sustains that mitochondrial ROS production increases with age because of an age-related decline of several ROS-scavenging enzymes and a decline in mitochondrial function. ROS increase leads to accumulation of mtDNA mutations, and a vicious cycle occurs because somatic mtDNA mutations impair respiratory chain function, which in turn results in a further increase in ROS production and accumulated oxidative damage to proteins, lipids, and DNA [5]. According to this theory, mitochondrial alterations have been extensively described in aging tissues of many organs [17]. In particular, a key reported feature of aging mitochondria was the increase in somatic point mutations and large deletions in the mitochondrial DNA (mtDNA) [18 19]. Since mtDNA encodes essential parts of the oxidative phosphorylation machinery, mutations of mtDNA cause oxidative phosphorylation dysfunction and a decline of cellular function. In addition, the number of mitochondria have been showed to decrease with age [20], thus contributing to the impaired ATP production and respiratory chain activity observed in the elderly [21]. Altered mitochondrial dynamics was reported during aging. In particular, the fusion protein Mfn2 is repressed in muscle during aging, determining the inhibition of mitophagy and of mitochondrial quality control that lead to the accumulation of damaged mitochondria [22].
Age-related decline in Lon expression and/or its proteolytic activity occurs in parallel with the accumulation of damaged proteins in rat liver mitochondria isolated from aged animals suggesting that Lon is a stress-response protein playing a key role in maintaining mitochondrial function. However, an increase of Lon protease expression was reported in rat heart [23] suggesting that its effect in aging may vary from one organ to another.

### Mitochondrial alterations in BC

The best characterized metabolic phenotype of tumor cells is the Warburg effect. Many years ago Otto Warburg observed that cancer cells actively metabolize glucose and produce an excess of lactate even in the

presence of oxygen, the so-called reverse Pasteur effect or aerobic glycolysis. He guessed that malignant cells should harbour defects in the respiratory chain of mitochondria and that cancer cells are able to increase the glycolytic rate to compensate the lower energy yield *per* single glucose. Also in BC the main energy source to sustain uncontrolled cells growth and proliferation is an aerobic glycolysis-dependent metabolism; indeed, BC cells display increased expression of genes coding for glycolysis, for the pentose phosphate pathway, and for fatty-acid synthesis [24] suggesting a deficit of mitochondrial activity in this cancer.

MtDNA mutations have been described in BC [25,26] in the form of point mutations, single-base deletions, and insertions in the non-coding D-loop region or in the coding regions for protein components of oxidative phosphorylation. In both human and rat bladder cancers, mtDNA exhibits a high rate of mutations. In particular, the repetitive sequences of mononucleotides within the mitochondrial genome are unstable and subjected to deletions. The tumorigenic role of mtDNA mutations in BC was demonstrated only for the *21-bp* deletion (from nucleotide position 15,642-15,662) in cytochrome B gene. This mutation was found in neoplastic tissue and urine of a BC patient [27]. Later on, it was shown that the overexpression of this mtDNA mutation generated increased ROS accompanied by increased oxygen consumption and lactate production and induced significant tumor growth *in vitro* and *in vivo* by triggering rapid cell cycle progression. Moreover, forced expression of this mutation induced mitochondrial proliferation and prevented apoptosis [28].

The high incidence of mtDNA mutations in BC suggests that mtDNA could play an important role in the process of carcinogenesis and could represent a valuable marker for early BC diagnosis. Like for prostate cancer, markers for early detection are eagerly awaited [29,30].

BC tissue has been found to display high levels of a marker of DNA oxidative damage, namely, 8'-hydroxy-2'-deoxyguanosine (OH8dG) [31] but also altered expression of some mitochondrial proteins. Lon protease expression level was found to be significantly higher in neoplastic compared to non-neoplastic tissue. Moreover, Lon expression was found to increase with tumor grade, being low in well differentiated (G1) BC, moderate in G2 BC, and high in poorly differentiated (G3) BC, with a dramatic difference between G2 and G3 tumors. It can be envisioned that Lon up-regulation may contribute to metabolic reprogramming observed in cancer by favoring the switch from a respiratory to a glycolytic metabolism that helps cancer cell survival in the tumor microenvironment.

The fusion protein *Mfn2* expression was found to be significantly lower in BC and its overexpression has been suggested to inhibit cell proliferation by arresting the transition of the cell cycle from the G1 to S phase, and to induce apoptosis [32]. Taking findings together, *Mfn2* gene seems to be a potential BC tumor suppressor gene that promotes apoptosis and inhibits the proliferation of BC cells.

Moreover, the mitochondrial transcription factor A (TFAM), a mitochondrial protein required for mtDNA replication, transcription and stability was found to be significantly increased in BC cells and to be directly related to tumor stage [33]. In the BC 5637 cell line, TFAM overexpression induced cell proliferation, migration and colony-forming ability, suggesting a role of TFAM in cancer progression.

If above-mentioned data suggest that mitochondrial dysfunctions may have prognostic role, no information is currently available regarding their potential predictive role, in other words, their ability to predict treatment response. In the last two decades, great efforts have been made to find molecular markers that can reliably predict BC response to available treatments. Most work has been done in the field on non muscle invasive bladder cancer (NMIBC), particularly high grade T1 disease [34-37] whose response to intravesical instillation of Bacille Calmette-Guerin is poorly predictable by standard clinical and pathological factors. Emerging evidence suggest that the combination of immunohistochemical markers is more effective than the single ones in predicting treatment response and disease outcome [38,39]. Case volume however remains an issue in this as well as in almost all fields; thus, studies with larger number of patients are eagerly awaited [40]. Moreover, like for other common urological diseases [41-43], the decision-making process should be tailored on patient conditions as well as wise clinical judgment.

Attempts have been made to identify molecular markers that can predict response also of MIBC to available treatment options, particularly systemic chemotherapy [41]. Markers of mitochondrial function are among novel putative molecular predictive markers [42]. More important, there are grounds to assume they could represent novel potential therapeutic targets [43], and we are working to assess whether they could contribute to modulation of that immune response which is demonstrated in other urological cancers [47,48].

## CONCLUSIONS AND PERSPECTIVES

In aging, the accumulation of ROS and somatic mtDNA mutations together with dysregulation of mitochondrial dynamics and mitochondrial quality control may induce mitochondrial functional decline contributing to age-related decline. Similarly, in BC the oxidative stress

related to smoking and to exposure to carcinogens may induce accumulation of somatic mtDNA mutations and alterations in mitochondrial dynamics and quality control, which may lead to mitochondrial dysfunction. These findings would support the hypothesis that age-related mitochondrial oxidative damage may reinforce and exacerbate the oxidative damage due to smoke and carcinogens and this may be one possible explanation for the increased risk of BC in elderly or even for its trend to recur in smokers [49].

If this is true, it is attractive to assume that improving mitochondrial function by antioxidant supplementation could be useful to prevent BC as well as to increase response to available treatments.

### Conflict of Interest

The authors declare no conflict of interest.

## References


1 Cumberbatch MGK, Jubber I, Black PC, et al. *Epidemiology of bladder cancer: a systematic review and contemporary update of risk factors in 2018*. Eur Urol 2018;74:784-95.

2 Lee CH, Yu HS. *Role of mitochondria, ROS, and DNA damage in arsenic induced carcinogenesis.* Front Biosci (Schol Ed) 2016;8:312-20.

3 Yalcin O, Karatas F, Erulas FA, et al. *The levels of glutathione peroxidase, vitamins A, E, C, and lipid peroxidation in patients with urothelial carcinoma of the bladder*. BJU Int 2004;93:863-6.

4 Badjatia N, Satyam A, Singh P, et al. *Altered antioxidant status and lipid peroxidation in Indian patients with urothelial bladder carcinoma.* Urol Oncol 2010;28:360-7.

5 Harman D. *The biologic clock: the mitochondria?* J Am Geriatr Soc 1972;20:145-7.

6 Sun N, Youle RJ, Finkel T. *The mitochondrial basis of aging*. Mol Cell 2016;61:654-66.

7 Murphy MP. *How mitochondria produce reactive oxygen species*. Biochem J 2009;417:1-13.

8 Chen X, Butow RA. *The organization and inheritance of the mitochondrial genome.* Nat Rev Genet 2005;6:815-25.

9 Musicco C, Cormio A, Calvarus, MA, et al. *Analysis of the mitochondrial proteome of cybrid cells harbouring a truncative mitochondrial DNA mutationin respiratory complex I.* Mol Biosyst 2014;10:1313-9.

10 Yoo SM, Jung YK. *A molecular approach to mitophagy and mitochondrial dynamics*. Mol Cell 2018;41:18-26.

11 Hamon MP, Bulteau AL, Friguet B. *Mitochondrial proteases and protein quality control in ageing and longevity.* Ageing Res Rev 2015;23:56-66.

12 Mishr P, Chan DC. *Metabolic regulation of mitochondrial dynamics*. J Cell Biol 2016;379:387-8.

13 Twig G, Elorza A, Molina AJ, et al. *Fission and selective fusion govern mitochondrial segregation and elimination by autophagy.* EMBO J 2008;433:446-73.

14 Arnoult D, Rismanchi N, Grodet A, et al. *Bax/Bak-dependent release of DDP/TIMM8a promotes Drp1-mediated mitochondrial fission and mitoptosis during programmed cell death*. Curr Biol 2005;2112:2118-26.

15 Gomes LC, Di Benedetto G, Scorrano L. *During autophagy mitochondria elongate, are spared from degradation and sustain cell viability.* Nat Cell Biol 2011;589-98.

16 Bota DA, Davies KJ. *Mitochondrial Lon protease in human disease and aging: including an etiologic classification of Lon-related diseases and disorders.* Free Radic Biol Med 2016;100:188-98.

17 Theurey P, Pizzo P. *The aging mitochondria.* Genes 2018;9:pii:E22.

18 Cormio A, Milella F, Vecchiet J, et al. *Mitochondrial DNA mutations in RRF of healthy subjects of different age.* Neurobiol Aging 2005;26:655-64.

19 Cormio A, Lezza AM, Vecchiet J, et al. *MtDNA deletions in aging and in non-mitochondrial pathologies.* Ann N Y Acad Sci 2000;908:299-301.

20 Herbener GH. *A morphometric study of age-dependent changes in mitochondrial population of mouse liver and heart*. J Gerontol 1976;31:8-12.

21 Short KR, Bigelow ML, Kahl J, et al. *Decline in skeletal muscle mitochondrial function with aging in humans.* Proc Natl Acad Sci USA 2005;102:5618-23.

22 Sebastián D, Sorianello E, Segalés J, et al. *Mfn2 deficiency links age-related sarcopenia and impaired autophagy to activation of an adaptive mitophagy pathway.* EMBO J 2016;35:1677-93.

23 Delaval E, Perichon M, Friguet B. *Age-related impairment of mitochondrial matrix aconitase and ATP-stimulated protease in rat liver and heart*. Eur J Biochem 2004;4559-64.

24 Massari F, Ciccarese C, Santoni M, et al. *Metabolic phenotype of bladder cancer*. Cancer Treat Rev 2016;45 46-57.

25 Fliss MS, Usadel H, Caballero OL, et al. *Facile detection of mitochondrial DNA mutations in tumors and bodily fluids.* Science 2000;287:2017-9.

26 Shakhssalim N, Houshmand M, Kamalidehghan B, et al. *Detection of mitochondrial DNA mutations in nonmuscleinvasive bladder cancer.* Genet Test Mol Biomarkers 2012;16:672-8.

27 Dasgupta S, Hoque MO, Upadhyay S, et al. *Mitochondrial cytochrome B gene mutation promotes tumor growth in bladder cancer.* Cancer Res 2008;68:700-6.

28 Dasgupta S, Hoque MO, Upadhyay S, et al. *Forced cytochrome Bgene mutation expression induces mitochondrial proliferation and prevents apoptosis in human uroepithelial SV-HUC-1 cells.* Int J Cancer 2009;125:2829-35.

29 Sanguedolce F, Cormio A, Brunelli M, et al. *Urine TMPRSS2: ERG fusion transcript as a biomarker for prostate cancer: literature review*. Clin Genitourin Cancer 2016;14:117-21.

30 Falzarano SM, Ferro M, Bollito E, et al. *Novel biomarkers and genomic tests in prostate cancer: a critical analysis.* Minerva Urol Nefrol 2015;67:211-31.

31 Wada T, Tanj N, Ozawa A, et al. *Mitochondrial DNA mutations and 8-hydroxy-2'-deoxyguanosine content in*

*Japanese patients with urinary bladder and renal cancers.* Anticancer Res 2006;26:3403-8.

[32] Jin B, Fu G, Pan H, et al. *Anti-tumour efficacy of mitofusin-2 in urinary bladder carcinoma.* Med Oncol 2011;28:S373-80.

[33] Mo M, Peng F, Wang L, et al. *Roles of mitochondrial transcription factor A and microRNA-590-3p in the development of bladder cancer*. Oncol Lett 2013;6:617-23.

[34] Sanguedolce F, Brunelli M, D'amuri A, et al. *Evolving concepts and use of immunohistochemical biomarkers in flat non-neoplastic urothelial lesions: WHO 2016 classification update with diagnostic algorithm.* Biomarkers 2018;23:305-14.

[35] Bufo P, Sanguedolce F, Tortorella S, et al. *Expression of mitotic kinases phospho-aurora A and aurora B correlates with clinical and pathological parameters in bladder neoplasms.* Histol Histopathol 2010;25:1371-7.

[36] Cormio L, Tolve I, Annese P, et al. *Retinoblastoma protein expression predicts response to bacillus Calmette-Guérin immunotherapy in patients with T1G3 bladder cancer.* Urol Oncol 2010;28:285-9.

[37] Cormio L, Sanguedolce F, Cormio A, et al. *Human epidermal growth factor receptor 2 expression is more important than Bacillus Calmette Guerin treatment in predicting the outcome of T1G3 bladder cancer.* Oncotarget 2017;11:25433-41.

[38] Cormio L, Tolve I, Annese P, et al*. Altered p53 and pRb expression is predictive of response to BCG treatment in T1G3 bladder cancer.* Anticancer Res 2009;29:4201-4.

[39] Sanguedolce F, Cormio A, Massenio P, et al. *Altered expression of HER-2 and the mismatch repair genes MLH1 and MSH2 predicts the outcome of T1 high-grade bladder cancer.* J Cancer Res Clin Oncol 2018;144:637-44.

[40] Kandasami SV, Mamoulakis C, El-Nahas AR, et al.; CROES URS Global Study Group*. Impact of case volume on outcomes of ureteroscopy for ureteral stones: the clinical research office of the endourological society ureteroscopy global study.* Eur Urol 2014;66:1046-51.

[41] Wollin DA, Joyce AD, Gupta M, et al. *Antibiotic use and the prevention and management of infectious complications in stone disease*. World J Urol 2017;35:1369-79.

[42] Cormio L, Preminger G, Saussine C, et al. *Nephrostomy in percutaneous nephrolithotomy (PCNL): does nephrostomy tube size matter? Results from the Global PCNL Study from the Clinical Research Office Endourology Society*. World J Urol 2013;31:1563-8.

[43] Cormio L, Gonzalez GI, Tolley D, et al. *Exit strategies following percutaneous nephrolithotomy (PCNL): a comparison of surgical outcomes in the Clinical Research Office of the Endourological Society (CROES) PCNL Global Study.* World J Urol 2013;31:1239-44.

[44] Sanguedolce F, Bufo P, Carrieri G, et al. *Predictive markers in bladder cancer: do we have molecular markers ready for clinical use?* Crit Rev Clin Lab Sci 2014;51:291-304.

[45] Sanguedolce F, Cormio A, Bufo P, et al. *Molecular markers in bladder cancer: novel research frontiers*. Crit Rev Clin Lab Sci 2015;52:242-55.

[46] Cormio A, Sanguedolce F, Musicco C, et al. *Mitochondrial dysfunctions in bladder cancer: exploring their role as disease markers and potential therapeutic targets*. Crit Rev Oncol Hematol 2017;117:67-72.

[47] Gigante M, Pontrelli P, Herr W, et al. *miR-29b and miR-198 overexpression in CD8+ T cells of renal cell carcinoma patients down-modulates JAK3 and MCL-1 leading to immune dysfunction.* J Transl Med 2016;14:84-9.

[48] Caratozzolo MF, Valletti A, Gigante M, et al. *TRIM8 anti-proliferative action against chemo-resistant renal cell carcinoma.* Oncotarget 2014;5:7446-57.

[49] Serretta V, Altieri V, Morgia G, et al.; Gruppo Studi Tumori Urologici (GSTU) Foundation. *Cigarette smoking status at diagnosis and recurrence in intermediate-risk non-muscle-invasive bladder carcinoma.* Urology 2013;81:277-81.