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A tale of sex-specificity in DNA damage response pathways: the paradigmatic story of TSPYL2

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Summary. Sexual dimorphism in cancer is still a poorly explored field but it is recognized that differences between males and females should depend not only on hormones and sex chromosomes, but also on molecular pathways generally considered to act equally in both sexes, such as the DNA damage response (DDR).
Here, we will describe and discuss the sex specific regulation and function of the DDR protein TSPYL2 in cancer cells. We will highlight how these disparities could contribute to the different cancer predisposition and therapy outcome of males and females and the importance of considering sex in both basic research and clinical practice.

Keywords. DNA damage response, sex chromosomes, cancer.

Sexual dimorphism plays an important role in many different human physiological processes and diseases, including cancer. In fact, it has been recently acknowledged that males are more prone than females to develop non-sex-specific tumors, affecting lung, colon, skin, bladder, liver and brain, and that men have a worst prognosis than women.[1]

Reasons for these differences may be delved into the different action of circulating reproductive hormones, but recently evidence of sex disparities are emerging also in cellular pathways, involved in tumor initiation and cancer progression. Indeed, molecular mechanisms that up to now were considered to act equally in male and female cells, such as regulation of senescence, angiogenesis, immunity and epigenetic modifications have been described as influenced by sex.[2,3] Surprisingly, sexual dimorphisms have been recently found also in the DNA damage response (DDR), a complex signalling cascade that cells have evolved to face DNA lesions and prevent the onset of genomic instability that can finally lead to cancer.[4] Defects in these pathways constitute a strength for cancer cells, but they also represent a vulnerability to be exploited for therapy, since DDR deficient cancer cells are more susceptible to DNA damaging agents than normal cells that can repair the lesions.[5]

Therefore, sex differences in these pathways may contribute to the different cancer predisposition of men and women and may influence their response to therapy, finally establishing an important starting point for the development of novel and personalized cancer therapy. Exactly for these reasons the study of sexual disparities in DDR pathways is extremely urgent.

Nowadays numerous evidence of sexual dimorphism has been found in some physiological functions of the DDR, like in telomere length maintenance, hormones expression regulation, mitochondrial DNA repair and oxidative stress response.[6]

Differently, only few examples of sex differences in response to genotoxic pharmacological treatments and in DDR activation have been reported and they are generally restricted to specific inactivation or increased mutation rate of DDR genes. For example, the *TP53* tumor suppressor gene has been found more frequently mutated in male cancers than in female,[7] while, on the contrary, mutations in *ATRX*, another tumor suppressor gene, are more frequent in females and demonstrated a protective role against cancer formation since they increase anticancer immunity.[8] Finally, another protein with important function in carcinogenesis is the tumor suppressor RB, that negatively regulates cell cycle progression. This protein has been found specifically inactivated in male glioma indicating a more rapid G1-S transit and promotion of cellular proliferation also in DNA damage conditions.[9]

Notably, in the manuscript recently published by Cardano et al. in *Cell Death and Disease*[10] it is reported for the first time the sex-specific regulation and function of testis specific protein Y encoded like 2 (TSPYL2) in response to DNA damage and these results describe a new sex-specific mechanism of DDR regulation characteristic of cancer cells.

TSPYL2 is a nuclear protein belonging to the testis specific nucleosome assembly protein superfamily, whose members share the presence of a nucleosome assembly protein (NAP) domain important for chromatin remodelling and transcription regulation.[11] TSPYL2 is encoded by an X-linked gene, and it is considered a tumor suppressor since reduced levels of this protein have been found in tumors and associated with poor prognosis. Recently, TSPYL2 was found to participate to the DNA damage response to genotoxic stress, by inhibiting the histone deacetylase SIRT1 and promoting the

activation of p300 acetyl-transferase, finally leading to p53 acetylation and apoptosis induction.[11]

In this new manuscript,[10] the authors demonstrate for the first time that TSPYL2 is maintained at low levels in unstressed conditions by murine double minute 2 (MDM2)-dependent ubiquitination and protein degradation. However, after DNA damage, TSPYL2 gene transcription is induced and the protein, no longer ubiquitinated by MDM2, accumulates in normal and female cancer cells.

On the contrary, no TSPYL2 accumulation can be observed in male cancer cells in response to DNA damage unless they lost the male specific Y chromosome during the oncogenic process. In fact, the authors demonstrate that the Y linked gene sex determining region Y (SRY), a transcription factor important for sex determination during development, is frequently re-expressed in cancer cells and sustains MDM2 protein levels both before and after DNA damage. As a consequence, after genotoxic stress, TSPYL2 continues to be degraded. However, these studies, performed in cancer cells, rely on model systems with different genetic backgrounds and therefore the defective TSPYL2 accumulation observed in male cancer cells may be due not only to the presence of the Y chromosome, but also to mutations in other genes or variability in the expression of other gene products. Exactly for this reason the behaviour of TSPYL2 after genotoxic stress is variable among the male cancer cell lines, and only studies performed in the same cell lines, depleted or not of the male specific chromosome, could reveal the real contribution of SRY and other Y-linked genes to TSPYL2 regulation after DNA damage. The mechanism regulating TSPYL2 protein levels in normal and female cancer cells strongly reminds that of p53 which, in unstressed conditions, is maintained at low levels by MDM2 dependent ubiquitination. Instead, after DNA damage, MDM2 is displaced from p53, becoming unable to ubiquitinate it, and p53 rapidly accumulates. Results obtained by Cardano et al.[10] therefore suggest that p53 and its regulators may be modulated in a similar manner and further confirm that MDM2 is able to control p53 function at multiple levels.

These findings also corroborate the involvement of TSPYL2 and the existence of sexual dimorphisms in DDR pathways and indicate that these disparities may contribute to the different tumor predisposition and response to chemotherapy of males and females. In fact, in non-transformed and in female cancer cells, the accumulated TSPYL2 restricts the proliferation of damaged and potentially dangerous cells, finally preventing genome instability and cancer progression. Since both radiotherapy and chemotherapy operate largely through DNA damage, we think that these studies will have high relevance to improve anti-cancer treatment in the future.

Moreover, in accordance with a more relevant TSPYL2 role in female than in male cells, Cardano et al. reported that TSPYL2 is more frequently mutated in female specific cancers than in male tumors and that low expression of this protein is associated with poor prognosis[10].

Importantly, this manuscript represents the first evidence of a Y specific gene participating to DDR pathways. In fact, the authors not only demonstrated that SRY is re-expressed in cancer cells where it modulates MDM2 protein levels, but they also showed that SRY expression is induced in response to treatment with etoposide, a topoisomerase II inhibitor.[10] These results indicate for the first time that the male specific chromosome may have important and previously unidentified roles in the response to DNA damage, possibly contributing to differences between males and females in tumor predisposition and chemotherapy outcome.

Nonetheless, these data are not completely unexpected since it has been recently established that the Y chromosome, generally considered a genetic wasteland, is responsible for sex differences in many different human diseases, such as cancer, Alzheimer, Parkinson and cardiovascular diseases.[12] It should be however noted that also the loss of Y chromosome, an event frequently observed in peripheral blood of aged males, is generally associated with increased predisposition to cancer formation and cardiovascular diseases.[13] These findings suggest that the male specific chromosome bears both oncogenes and tumor suppressor genes and could have tissue- and cell-specific functions.

Therefore, the results obtained by Cardano et al.[10] indicate Y chromosome involvement in both the DDR and cancer, suggesting that more attention should be given to the male specific chromosome and to sex, as a biological variable, in both basic research and clinical practice. Indeed, the sex of patients is not considered during cancer screenings protocols and therapy choice, and this could lead to delay in diagnosis and increased toxicity during therapy, associated with poor quality of patients' life and unnecessary costs for the national health systems. Hence, the contribution of Y chromosome alterations to tumorigenesis, if confirmed, will constitute an important information for the national health systems, suggesting that men should be periodically checked for Y chromosome status and eventually enrolled in cancer screening at younger age than women. Moreover, the disparities in therapy sensitivity of males and females could indicate that different amounts and types of treatments should be administered to patients depending on their sex and Y chromosome status, reducing costs and negative side effects, and improving patients' quality of life. The pursuit of these considerations will enable us to achieve in the future the same clinical outcome for both male and female patients.

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