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REVIEW ARTICLE

MDM4: What do we know about the association between its polymorphisms and cancer?

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Abstract

MDM4 is an important p53-negative regulator, consequently, it is involved in cell proliferation, DNA repair, and apoptosis regulation. *MDM4* overexpression and amplification are described to lead to cancer formation, metastasis, and poor disease prognosis. Several *MDM4* SNPs are in non-coding regions, and some affect the *MDM4* regulation by disrupting the micro RNA binding site in 3'UTR (untranslated region). Here, we gathered several association studies with different *MDM4* SNPs and populations to understand the relationship between its SNPs and solid tumor risk. Many studies failed to replicate their results regarding different populations, cancer types, and risk genotypes, leading to conflicting conclusions. We suggested that distinct haplotype patterns in different populations might affect the association between *MDM4* SNPs and cancer risk. Thus, we propose to investigate some linkage SNPs in specific haplotypes to provide informative *MDM4* markers for association studies with cancer.

Keywords MDMX · HDMX · SNP · Haplotype · Tumor

Introduction

MDM4 (mouse double minute 4), also known as *MDMX* and *HDMX,* is in 1q32 loci of the human genome [1] which encodes a protein capable of forming a heterodimer with *MDM2* (mouse double minute 2) [2]. *MDM2* is an E3 ubiquitin ligase that binds to the guardian of the genome p53 promoting its proteasomal degradation. This activity can be enhanced by *MDM4-MDM2* complex formation [3]. *MDM2* is a paralogous gene of *MDM4*, and both proteins present 33% identity in humans. These genes emerged after the ancestral *MDM* duplication in primitive vertebrates. The ancestral protein was more *MDM2*-like, while *MDM4* evolved to lose its ubiquitin ligase activity [4]. Under normal conditions, these two proteins have autoregulatory feedback, a mechanism in which p53 can activate *MDM2* transcription [5], and the *MDM2-MDM4* heterodimer activity regulation is responsible for maintaining low levels of p53 when this protein is not necessary, preserving intracellular homeostasis [6]. During cellular stress, *MDM2* promotes self-degradation and *MDM4* degradation, leaving p53 free to activate transcription factors, promoting DNA repair, cell cycle arrest, and apoptosis. Thus, the p53-*MDM2-MDM4* regulatory axis alterations are mainly known to promote cancer [7].

The central regulator of the p53 pathway is *MDM2*; however, *MDM4* participates in the p53 level regulation in different ways (Fig. 1). The p53 inhibition occurs by direct *MDM4*-p53 protein binding (Fig. 1A), which abolishes the tumor suppression from its function, and blocks its transcriptional activity [8, 9]. MDM4 stabilizes *MDM2*, enhancing p53 ubiquitination and its proteasomal degradation by forming the *MDM2-MDM4* heterodimer (Fig. 1B) [10, 11]. The *MDM2-MDM4* heterodimer can promote p53 synthesis during genotoxic stress acting as IRES (Internal Ribosome Entry Site) transacting factors to activate p53 mRNA

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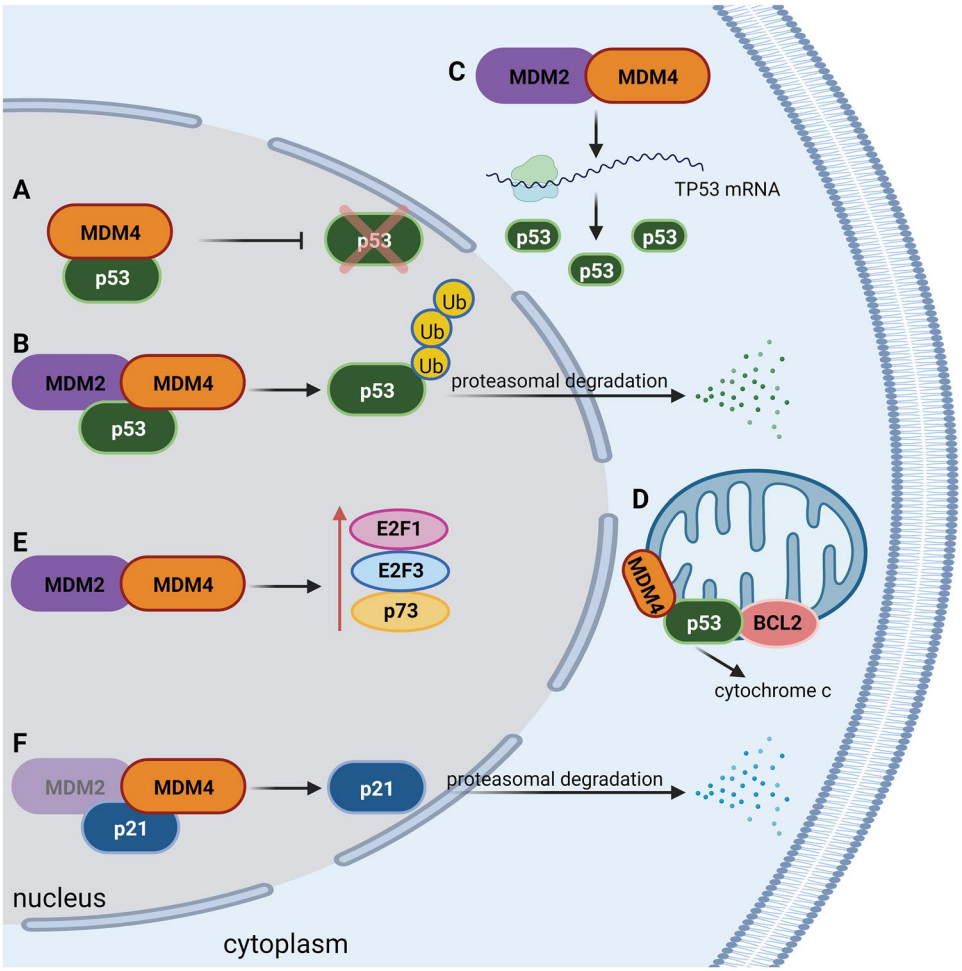

Fig. 1 MDM4 interaction with p53 and other proteins of cell cycle regulation. MDM4 is involved in some steps in the p53 pathway and other cell cycle control pathways. MDM4 can act alone or in a heterodimer with MDM2. MDM4 can directly bind and inhibit p53 activity **A** and enhances the p53 proteasomal degradation forming an MDM4-MDM2 heterodimer **B** In genotoxic stress conditions, MDM4 can act as an IRES transacting factor to increase p53 mRNA **C** and facilitates the interaction between p53 and BCL2 in mitochondria to promote apoptosis **D** The MDM4-MDM2 heterodimer interacts with the E2F protein family **E** and MDM4, alone or in cooperation with MDM2, mediates p21 proteasomal degradation to induce cell cycle progression **F** Created with *BioRender.com*

translation (Fig. 1C) [12]. Furthermore, a contrasting activity of MDM4 has been described, which requires further investigation, as a facilitator of the interaction between p53 and BCL2 (B-Cell Leukemia/Lymphoma 2) after genotoxic stress by promoting the displacement of p53 phosphorylated in Ser46 to mitochondria, releasing cytochrome c in the cytoplasm, and signaling apoptosis (Fig. 1D) [13]. In a p53-independent manner, MDM2-MDM4 heterodimer acts as a cell cycle promoter maintaining high levels of transcriptional factors E2F1, E2F3, and p73 when wild-type p53 is absent (Fig. 1E) [14]. *MDM4*, alone or in cooperation with MDM2, can promote the transition from G1 to the early S phase, binding directly to p21 to mediate its proteasomal degradation (Fig. 1F) [15]. These *MDM4* activities can contribute to cancer development, and some *MDM4* transcripts responsible for encoding proteins are related to different cancer types [16].

Tumor development depends on pathways frequently deregulated in cancer which often play essential roles in promoting aberrant splicing [17]. *MDM4* protein diversity is created by alternative splicing (Fig. 2), and the most described isoforms in cancer studies are *MDM4*-FL (full-length) and *MDM4*-S (short form). *MDM4*-FL is more stable than the latter [18], and it has the complete *MDM4* protein structure with all four conserved regions: the N-terminal portion with p53 binding domain, the acid domain, zinc finger, and RING finger domain in the C-terminal portion (Fig. 2A) [2]. The skipping of exon 6 results in a frameshift and a premature stop codon that encodes a short carboxy-truncated *MDM4* protein (*MDM4*-S) (Fig. 2B) containing the p53 binding domain and a few residues in the C-terminal portion [19]. Some authors described *MDM4*-S as presenting a higher affinity with p53 when compared with *MDM4*-FL [20], and *MDM4*-S presents higher expression levels than *MDM4*-FL in tumor cells [21–23]. *MDM4*-S expression is related to more aggressive p53 mutated cancer, and the *MDM4*-S/*MDM4*-FL ratio could be useful as a potential prognostic biomarker [22, 24, 25]. Some *MDM4* isoforms are also related to cancer: *MDM4*-211(Fig. 2C) mRNA is highly expressed in thyroid tumor cell line (ARO) [26], and it is frequently expressed in papillary thyroid carcinoma samples, while MDM4-FL is downregulated [27]; *MDM4*-A (Fig. 2D) mRNA is significantly more expressed in human melanoma samples than *MDM4*-FL, and its

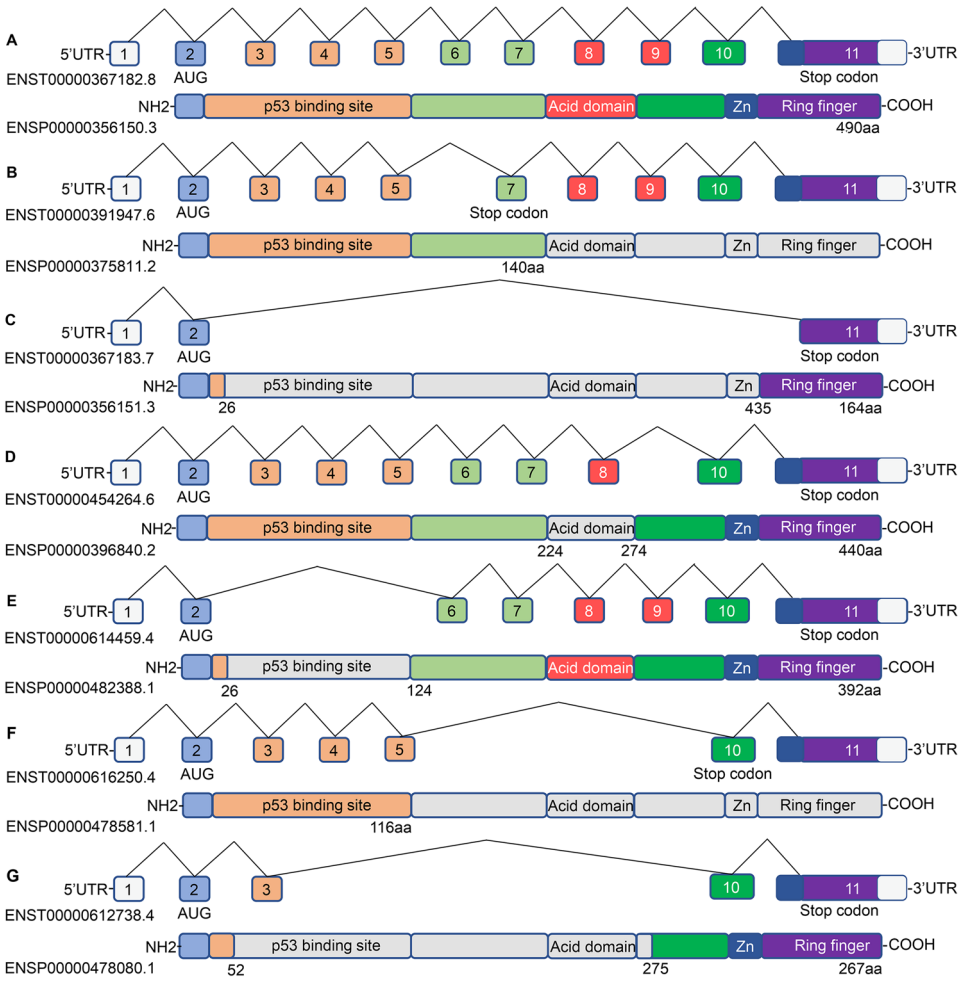

Fig. 2 MDM4 isoforms related to cancer. The isoforms transcripts and proteins (represented by letters A to G) are named by Ensembl codes (ENST and ENSP, respectively). Each conserved protein domain and its respective transcript exons have the same color, i.e., p53 binding domain (orange), acid domain (red), zinc finger (Zn) (blue), RING finger domain (purple) and missing domains (gray). The *MDM4* UTR portions are in white color. The MDM4 isoform sizes is shown by amino acids (aa) number. The MDM4-FL is the full-length isoform with 490 aa with all exons and conserved domains **A** MDM4-S isoform has only the p53 binding domain, and its transcript skips exon 6, creating a frameshift and a premature stop codon in exon 7 **B** MDM4-211 isoform presents only the RING finger domain after its exons 3 to 10 are removed **C** MDM4-A isoform has an incomplete acid domain since its transcript skips exon 9 **D** MDM4-G isoform has no p53 binding domain, and its transcript skips exons 3 to 5 **E** MDM4-Alt1 isoform has only the p53 binding domain since its transcript skips exons 6 to 9, with a premature stop codon in exon 10 **F** MDM4-Alt2 isoform has only the zinc finger and RING finger domains after its exons 4 to 9 are removed **G**

expression is correlated to poor survival [28]; *MDM4*-A and *MDM4*-G (Fig. 2E) were first isolated from an ovarian cancer cell line (C33A), and they both might affect p53 activity. *MDM4*-G stabilizes MDM2, while *MDM4*-A inhibits p53 [29]. *MDM4*-ALT1 (Fig. 2F) and *MDM4*-ALT2 (Fig. 2G) expressions were described by Chandler et al. [30] in tumor breast cell lines (MCF-7), and *MDM4*-ALT2 was associated with high metastatic risk of rhabdomyosarcoma [31]. However, *MDM4* isoforms need further investigation to evaluate their roles in carcinogenesis.

Alternative splicing is also a fundamental process to regulate gene expression. However, it is also known that *MDM4* is amplified or overexpressed in many human cancers, such as glioma, lung, colon, breast, retinoblastoma, prostate, squamous cell carcinoma of head and neck, gastric, and acute myeloid leukemia (AML) [32–40]. Previously, it was observed that spontaneous tumorigenesis in transgenic mice with wild-type p53 was induced by *MDM4* overexpression [41]. *MDM4* and *MDM2* overexpression were correlated with an increase in circulating tumor cells in triple-negative breast cancers in a mouse model [42], while p53 mutations were correlated with *MDM4* amplification and *MDM2* overexpression in primary breast tumors [43]. Interestingly, *MDM4* expression can be controlled by

micro RNAs (miRNAs). MiRNAs are non-coding RNAs with approximately 30 base pairs that regulate protein levels post-transcriptionally [44]. Some miRNAs can bind in *MDM4* mRNA 3'UTR, resulting in low levels of *MDM4* and p53 function release [45–47]. Some miRNAs have an affinity with *MDM4* mRNA affected by SNPs (Single Nucleotide Polymorphisms) located on 3'UTR [48–51]. Furthermore, *MDM4* has an assortment of SNPs that can be used as biomarkers for different types of tumors since some miRSNPs interfere with gene expression and may contribute to clinical outcomes affecting cancer susceptibility [52, 53]. Herein, we reviewed the association studies between *MDM4* SNPs and cancer, focusing on the controversy of those SNPs previously associated with cancer risk, solid tumors prognosis, and cancer survival. We propose to investigate some linkage SNPs in specific haplotypes that might help future association studies with *MDM4* and cancer.

MDM4 SNPs associated with cancer risk

The *MDM4* roles in tumorigenesis and cancer aggressiveness have been studied for the last few years, and some findings from *MDM4* SNPs association studies with cancer in different populations can create insights in terms of understanding the *MDM4* allelic establishment of cancer susceptibility. Interestingly, all *MDM4* SNPs observed in these studies are in non-coding regions, except the rs116197192, located in exon 7 (Supplementary Table 1). The most studied *MDM4* SNP, the rs4245739 (C > A), is in 3'UTR. It was predicted to change the affinity of three miRNAs: miR191-5p, miR-887, and miR-3669 [54]. This miRSNP disrupts miRNA binding sites and reduces the *MDM4* oncogenic effects. Indeed, the allele rs4245739-C significantly affects the binding of miR-191-5p and miR-887 and decreases *MDM4* expression in heterozygous genotype (A/C), while no effect was observed in homozygous genotype (A/A) in different cancer cell lines [49]. Regarding that, the localization of SNPs in distinct gene regions as promoter, exons, introns, and UTRs modulates gene expression in different ways, for example, by altering the splicing machinery and/ or long non-coding RNA (lncRNAs) binding sites and affects mRNA expression, which may play a functional role in cancer susceptibility [55]. However, most studies about *MDM4* SNPs and their association with cancer do not provide information concerning their impact on gene expression and its alternative splicing. In addition, the genetic background of the analyzed population and the cancer characteristics might explain the conflicting results between functional and association studies. The rs4245739 A/A genotype was associated in the Norwegian population with the breast cancer increased risk, while the same genotype was associated with the ovarian cancer reduction risk in this population (Supplementary Table 1) [56, 57].

However, most studies described the rs4245739 A/A genotype or rs4245739-A allele as positively associated with many cancer types (ovarian, esophageal, breast, lung, oropharynx, and colorectal) in a diversity of European and Asian populations [46–48, 58–64]. Recently, Chen et al. [65] and Wang et al. [66] confirmed by meta-analysis the association between rs4245739-C and the reduction of cancer risk in the Asian ancestry population. On the other hand, few studies demonstrated that the rs4245739 C/C genotype is associated with increased cancer risk in different populations [67], but most of them correlated this genotype with negative estrogen receptor (ER-) breast cancer subtype in other populations [68, 69].

Some studies evaluated whether the *MDM4* SNPs influence clinicopathological cancer aspects, such as overall survival (OS), disease-free survival (DFS), disease recurrence, and tumor aggressiveness. Specifically, the rs4245739 C/A and C/C genotypes in NSCLC (Non-Small Cell Lung Cancer) and colorectal cancer were significantly associated with better OS [46, 63]. On the other hand, the ovarian carcinoma patients with rs4245739 A/A genotype presented an increased death risk and a significant reduction in their OS compared with C allele carrier patients [48]. After chemoradiation in the HPV16 + patients with SCCOP (Squamous Cell Carcinoma of the Oropharynx), it was observed that patients with rs4245739 C/C and A/C genotypes had the risk of overall death, death due to disease, and disease recurrence reduced in comparison to the patients with rs4245739 A/A [62]. This *MDM4* polymorphism is extensively studied since its 3'UTR is functionally related to altering target recognition of miRNAs by disrupting sequence complementarity [48]. As a regulatory region, the 3'UTR is indispensable for regular gene expression, and the *MDM4* rs4245739 polymorphism could affect its protein translation. As MDM4 forms an MDM2 complex that promotes p53 degradation, resulting in cell cycle deregulation and creating a carcinogenesis environment, this *MDM4* SNP has a suggestive relevance in this process [49].

In addition to this SNP, other associated SNPs are located in *MDM4* UTRs but are no longer functionally analyzed. The genotype A/A of 3'UTR rs11801299 (G > A) was associated with lower OS, higher tumor invasion, and poor tumor differentiation in retinoblastoma Chinese patients in comparison to G/G genotype [70]. The G/A genotype was associated with gastric cancer susceptibility in the Chinese population [38]. Different findings in the American non-Hispanic population association studies reinforce the importance of population background genetic importance. In this population, the SCCOP patients with rs11801299 G/G genotype had worse DFS in comparison to patients with rs11801299 A/A or A/G genotypes as well as with *MDM4* 3'UTR rs10900598

(G > T/ C > A) T/T + T/G genotypes in comparison with G/G genotype [71]. The rs10900598 C/C genotype was associated with reduced NSCLC risk in the Chinese population, while a genotype synergic effect was demonstrated between rs10900598 C/C and rs4245739 C/C, both polymorphisms located in *MDM4* 3'UTR. The NSCLC patients with these genotypes had better OS than those without these genotypes' combinations [46].

The functionally less known and analyzed SNPs in association studies were *MDM4* 5'UTR rs10900594 (G > C) and 3'UTR rs12039454 (T > C). These SNPs were analyzed together with the intronic *MDM4* rs2369244 (G > C) in one single association study with breast cancer risk in the Ashkenazi Jewish population (Supplementary Table 1) [52]. Additional investigations will be required to determine the biological relevance of these SNPs. However, it has been demonstrated that the promoter region SNPs affect gene expression by altering promoter activity, transcription-factor binding, DNA methylation, and histone modification [72–74]. Indeed, *MDM4* polymorphisms located in the 5'UTR region, such as rs10900594 and rs4252668 (T > C), might play an important role in its transcription that interferes with MDM4 oncogenic activity. In fact, Reincke et al. [75] suggested that the rs4252668 variant may have a regulatory function for being located at 5'UTR in *MDM4*. According to the SNP Nexus portal, rs4252668 is in a CpG island (CGI) with 69.3% C/G content and 358 base pairs in length [76]. DNA methylation occurs primarily in the CGIs of the promoter region. Therefore, SNPs in the promoter region, such as *MDM4* rs4252668-T, might alter DNA methylation status, histone acetylation, chromatin modification, and gene silencing. Thus, epigenetic modifications are associated with disease and cancer development [77, 78]. In a study developed by Oliveira Reis et al. [79], the *MDM4* polymorphisms rs4252668-T and rs116197192-G were associated with retinoblastoma increased risk in the Brazilian population. Interestingly, all patients had the rs4252668 T/T genotype, while the C/C genotype was at a higher frequency in the control group.

The *MDM4* rs116197192 (A > G) in exon 7 gives rise to D153G substitution located within a predicted casein kinase II (CK2) site. Both protein kinases, CK1 and CK2, participate in various cellular processes, including DNA repair and cell cycle control, and phosphorylate Ser or Thr residues [75]. Phosphorylation on MDM4 Ser289 by CK1α increases its association with p53 with a profound impact on its p53 inhibition [80], but there is no report of *MDM4* phosphorylation by CK2 at Thr150 or other sites [81]. According to TarBase v.8 [82], rs116197192 and rs4252668 are in miRNAs binding sites, and these interactions promote the downregulation of *MDM4* in different cell lines and tissues. The allele change of these polymorphisms might increase cancer risk by modifying the regulation site, upregulating *MDM4* expression, and inhibiting p53 activity. However, the SIFT portal [83] shows a prediction that the effect of change A to G in rs116197192 (D153G) is tolerated in the *MDM4* function.

The polymorphism MDM4 rs4252707 (G > A) in intron 8 might modulate mRNA splicing activity and impact the production of the splice variants without exon nine as *MDM4*-A isoform. The rs4252707-A was associated with increased risk for non-glioblastoma glioma in the European and Chinese populations [84, 85]. The rs1563828 (A > G/ T > C) is an intronic variant in which T/T and A/A genotypes were described as associated with earlier ovarian tumor onset [52], higher breast tumor aggressiveness [53], and 1.3-fold increased risk of lymphatic vessel infiltration in breast cancer patients compared with C/C genotype (Supplementary Table 1) [61]. In contrast, Morvan et al. [86] described the rs1563828 A/A breast cancer patients with higher PFS (Progression-Free Survival) compared with G/G and A/G patients after chemotherapy.

The *MDM4* rs1380576 (G > C) located in intron 1 is one of the *MDM4* SNPs that usually is analyzed in association studies with cancer (Supplementary Table 1). The genotype C/C was associated with prostate cancer increased aggressiveness in the German population [35] and positive estrogen receptor (ER +) breast cancer increased risk in the Lithuanian population [64]. A protective effect was observed with the rs1380576 G/G genotype, which was associated with a significant breast cancer decrease in the Iranian population [87], lower retinoblastoma tumor aggressiveness [70], and gastric cancer reduction in the Chinese population [38]. The HapMap datasets from Asian and Chinese populations associated this genotype with lower *MDM4* mRNA expression [38]. Interestingly, the rs1380576 G/G genotype was associated with many types of cancer risk reduction in the Asian population [65], and the C/C genotype was associated with reduced cancer risk compared with the G/G + G/C genotypes in the Asian ancestry population (Supplementary Table 1) [66]. Considering that high *MDM4* expression may result in low levels of p53 and the activation of different cell cycle-promoting proteins [14, 42], this associated polymorphism could interfere with the reduction of *MDM4* level and, consequently, it might contribute to reducing cancer development.

The *MDM4* expression might be affected by quantitative trait loci alleles (eQTL). A particular *MDM4* downstream intergenic variant in 1q32.1, the rs12133735-G allele (G > T), was related to a lower *MDM4* expression; however, it was associated with increased risk for lung, and oropharyngeal cancer in the European population according to GWAS meta-analysis study [88]. The additional information extracted from the GTEx portal (ENSG00000198625.12) listed the aforementioned *MDM4* SNPs associated with cancer risk and a significant *MDM4* eQTL effect. The

rs4245739, rs1380576, rs1563828, rs10900598, rs10900594, rs2369244, and rs12039454 are related to a negative *MDM4* eQTL effect in several tissues, except rs11801299, which effect was seen only in blood. No eQTL impact was found for rs4252668 and rs116197192. According to NESDA NTR Condicional eQTL Catalog, the allelic effects in the blood of the top associated SNPs (rs4245739-C, rs2369244-G, rs10900594-G, and rs1380576-G) have significant negative *MDM4* eQTL. In contrast, rs12039454-T, rs10900598-G, rs1563828-A, and rs11801299-A have a positive *MDM4* eQTL effect in European ancestry individuals [89]. This data could explain why some alleles are associated with increased cancer risk as they can increase *MDM4* expression and downregulate the p53 pathway.

Lack of association between *MDM4* SNPs and cancer risk

The association studies represent an essential step in defining disease-mediating genetic variants. However, some studies failed to show an association between *MDM4* SNPs and cancer risk or survival as a consequence of population-specific genotype frequencies, differences due to genetic background, and solid tumor heterogeneity (Supplementary Table 2) [57, 90–92]. Despite the potential for improving the ability to detect susceptibility/protection SNPs, the inconsistency of association data is still a feature of association studies [93–97]. One of the reasons for this problem might be the small sample size, as observed in some association studies herein analyzed [46, 85, 91, 92, 98]. The strategy to explore a large group of *MDM4* SNPs in a genome regional association study that has never been studied before in the Chinese population was useful in informing allele and haplotype descriptions, despite the absence of significant results observed for seven *MDM4* SNPs (Supplementary Table 2) [46, 85].

The cancer risk associations detected for some *MDM4* SNPs herein studied were not reproducible in other association studies with different cancer types and populations (Supplementary Tables 1 and 2). The ethnic diversity leading to population stratification can bear significantly on the power of an association study. In the same vein, the association studies between *MDM4* rs4245739 (C > A) and lung, colon, and prostate cancer risks showed different conclusions in the Norwegian [56] population in comparison to association studies with Asian ancestry populations.

A further explanation source of nonreplicable results might be the small effect of many genetic factors contributing to cancer formation. For instance, the impact of HPV infection, one of the environmental risk factors for oropharyngeal, head, and neck cancer development, could be stronger than the exclusive presence of some *MDM4* genotypes. In fact, Wang et al. [92] showed a significant association between *TP53, MDM2*, and *MDM4* SNPs and oral cancer risk only for HPV16 + patients when the risk genotypes of these three genes are together. No association was observed for each *MDM4* SNPs (rs11801299, rs10900598, rs1380576) in HPV16 + or HPV16− patients. Thus, the relative risk of only *MDM4* genetic variants might be small [99].

MDM4 SNPs in linkage disequilibrium and haplotypes

The characterization of multiple SNPs (marker alleles) on the same chromosome, which tends to be inherited together, is termed haplotype. The association analysis based on haplotypes may provide more power and accuracy in disease gene mapping than those based on single markers [100]. The linkage disequilibrium (LD) analysis based on underlying haplotypes varies significantly in different populations and reflects the pattern of inheritance over evolution [101]. Based on the *MDM4* SNPs single data found in the association studies with cancer risk listed in Supplementary Tables 1 and 2, we inferred the haplotype configurations of 18 total SNPs in three different populations: Euro-American (CEU), Han Chinese (CHN), and Afro-American (ASW) using the linkage disequilibrium information from LD link tools [102].

Accordingly, we observed 12 different *MDM4* haplotypes over three populations (Fig. 3A). The phylogenetic relationships within 12 *MDM4* haplotypes were performed using a set of haplotypes dissimilarities to construct a hierarchical cluster (Fig. 3B). The most similar clusters were built by *Ward's* minimum variance method and the resulted phylogenetic haplotype tree demonstrates 3 major clusters (Fig. 3B). Specifically, haplotype 2 differs from 11 to 12 ancestral haplotypes by 11 *MDM4* SNPs alleles (Fig. 4). Haplotype 12 (5.7%) is unique to the ASW population, and it is represented in the smallest cluster (Fig. 3B) in which the haplotypes 1 and 4 diverge by rs12133735 allele (Fig. 4). The haplotype 1 is the most frequent in the CEU and CHN population and, the third most common in the ASW population, while the haplotype 4 is observed only in the CEU population (Fig. 3A). Interestingly, the CHN population showed more haplotype combinations than CEU and ASW populations, since haplotypes 9, 10, and 11 are exclusive to the CHN population and these haplotypes are represented together with the haplotype 3 in the second haplotype cluster (Fig. 3B). These two clusters separated the CHN and the ASW populations according to the *MDM4* SNPs alleles. One of the differences between these two clusters (ASW x CHN) is the eQTL *MDM4* SNP rs11801299-A allele, which was

A

SNP	CEU	CHN	ASW
1	42.9	32.2	13.1
2	30.8	4.6	28.7
3	19.2	28.9	6.6
4	2	0	0
5	1.5	1.2	2.5
6	1	0	0
7	0	16.4	32.8
8	0	8.2	5.7
9	0	2.9	0
10	0	2.4	0
11	0	1.2	0
12	0	0	5.7
Others	2.6	2	4.9

B

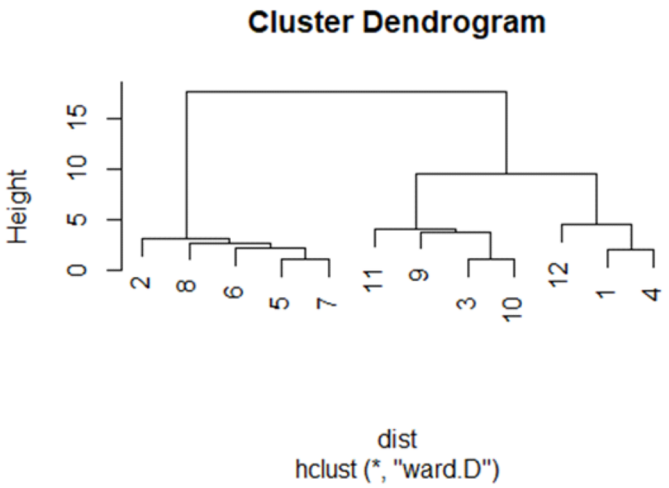

Fig. 3 Haplotypes frequencies in each population: CEU population (Utah residents with Northern and Western European ancestry), the CHN (Han Chinese in Beijing and Southern with East Asian ancestry), and the ASW population (African Ancestry in the Southwest United States) **A** and the dendrogram grouped by Euclidian distance and Ward.D linkage method as Hierarchical Clustering **B** using the packages dist() and hclust() in R programming language. The Euclidian distance (Height) organizes the haplotypes according to similarity relations to obtain a classification, while Ward's method creates groups minimizing the variation within clusters. Haplotypes and LD correlation analysis were generated by the LD Link tools [102]

previously related to positive *MDM4* expression [89] and herein it was found as a cancer risk in the CHN population.

The third cluster is composed of more haplotypes (haplotypes 2, 8, 6, 5, and 7) with distinct frequencies along the three populations analyzed (Fig. 3A, B). The most frequent haplotype in this cluster is haplotype 7, with 32.8% in the ASW population and 16.4% in the CHN population, while absent in the CEU population. Interestingly, haplotype 7 is the only one that has the *MDM4* SNP rs3014610-T allele (A > T) (Fig. 4). According to ENCODE portal [103], this SNP is related to a region with post-translationally modified histones (H4K20me1, H3K36me3) in tumor cell lines. Considering its high frequency in the ASW and CHN populations (Fig. 3A), haplotype 7 could be protective against cancer development. However, the rs3014610 intronic variant needs further investigation as it is not associated with cancer risk in the Chinese population (Supplementary Table 2). Haplotype 6 is unique to the CEU population, and it diverges from haplotypes 5, 7, and 8 by *MDM4* SNPs rs4252668, rs16853949, rs10900594, and rs1380576 alleles. Haplotype 6 has rs4252668-C, and haplotype 8 has rs16853949-A alleles (Fig. 4), which are uncommon in the three populations (Supplementary Table 6) and might be related to conflicted association studies results herein observed (Supplementary Table 2). Additionally, the rs4252668 is in the transcriptional factors binding site and the rs16853949 is in regions with post-translationally modified histones in tumor cell lines, and both might impact *MDM4* expression (ENCODE portal [103]). The last two SNPs are related to histone epigenetic modifications and eQTL effects that might affect cancer susceptibility directly or indirectly. Haplotype 2 is very common in the CEU and ASW populations and less frequent in the CHN population (Fig. 3A). The difference between haplotype 2 and the other haplotypes is the presence of *MDM4* SNP rs4245739-C allele (Fig. 4), which functionally disrupts miRNA binding site, and has a significant negative *MDM4* eQTL effect. Haplotype 2 is less frequent in the CHN population, and its distribution might affect the association studies in populations with different genetic backgrounds.

The haplotype-based association methods are generally more powerful than methods based on single markers since it exploits LD information from multiple markers. D' and r^2 are LD measurements based on a pair of markers. LD patterns and haplotype frequencies diverge significantly in different populations. Indeed, we observed in the CEU

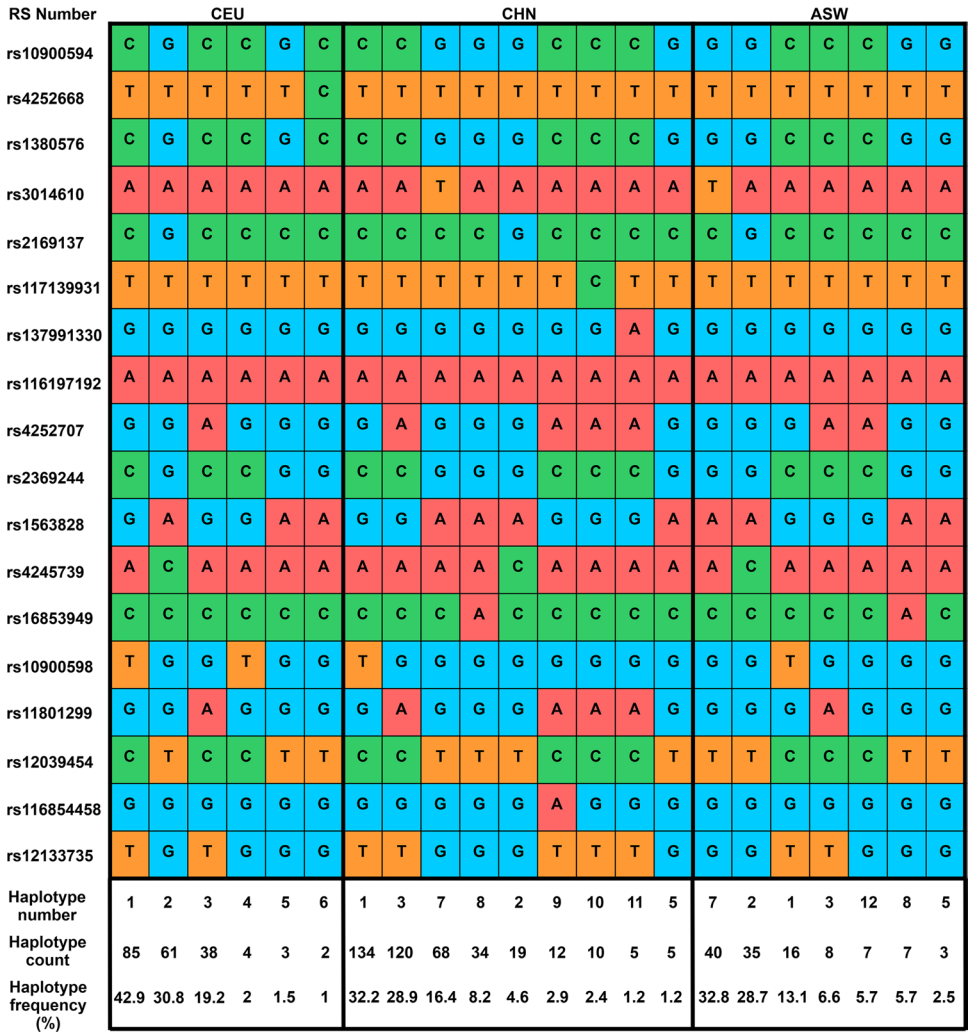

Fig. 4 *MDM4* SNPs and haplotypes in different populations. The *MDM4* haplotypes frequencies were calculated for the CEU population (Utah residents with Northern and Western European ancestry), the CHN (Han Chinese in Beijing and Southern with East Asian ancestry), and the ASW population (African Ancestry in the Southwest United States). Haplotypes and LD correlation analysis were generated by the LD Link tools [102]

and the CHN populations (Supplementary Tables 3 and 4, respectively) a strong linkage disequilibrium between *MDM4* rs4252707 and rs11801299 ($D' = 1$) and low LD in the ASW population (Supplementary Table 5). Haplotype 12 might result from this weak LD observed in the ASW population. Herein, we listed studies with no association between rs11801299 and cancer risks in the American non-Hispanic population (Supplementary Table 2).

The *MDM4* rs4245739 is in strong LD with rs2169137 in the CEU and CHN populations. In the ASW population, these SNPs are highly correlated ($r^2 = 0.924$). Interestingly, Haplotype 2 is characterized by the rs4245739-C and rs2169147-G alleles that were found in low frequency in the CHN population compared to the two other populations (Fig. 3A). The *MDM4* SNP rs10900594 is strongly linked with rs1380576 in the CEU population and has a high correlation in the CHN and the ASW populations ($r^2 = 0.989$ and $r^2 = 0.92$, respectively). Curiously, both protective alleles (rs10900594-G and rs1380576-G) are in the same haplotype combination with the susceptibility alleles (rs1563828-A and rs12039454-T) located in the no coding region (intron 10 and 3'UTR, respectively), forming two contrasting blocks with a significant *MDM4* eQTL SNPs (Fig. 4). The two first alleles are related to a negative eQTL effect and, the two last alleles have a positive *MDM4* eQTL effect. It seems that along the length of *MDM4,* there are different ways to control gene expression since rs1380576 and rs12039454 SNPs are located at binding sites for various transcription factors. Additionally, the posttranslational modifications of histones alter chromatin structure, impacting transcriptional factors binding and increasing the oncogenic process. The histone H3 tail lysine and arginine residues suffer covalent modifications, like acetylation and methylation. Both modifications might occur on these four SNPs described above: H3K27me3, H3K4me1, H3K36me3, and H3K27ac (ENCODE portal [103]). These epigenetic modifications could activate an oncogene, such as *MDM4*, thus providing oncogenic reprogramming in tumor cells.

Conclusions

The results indicated that the *MDM4* association studies with solid tumors suffer from the influence of the differences in population genetic structure. Here the single SNPs listed were useful in constructing the haplotype structures. The level of linkage between some eQTL alleles and SNPs was also useful in exploiting the *MDM4* polymorphism in cancer. Our results suggest some additional haplotypes to refine the association studies results.

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Data availability All data analyzed during this study are included in this published article and its supplementary information file.

Declarations

Conflict of interest The authors declare that no conflicts of interest exist.

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