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Characterization of cell cycle, inflammation, and oxidative stress signaling role in non-communicable diseases: Insights into genetic variants, microRNAs and pathways

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ABSTRACT

Non-Communicable Diseases (NCDs) significantly impact global health, contributing to over 70% of premature deaths, as reported by the World Health Organization (WHO). These diseases have complex and multifactorial origins, involving genetic, epigenetic, environmental and lifestyle factors. While Genome-Wide Association Study (GWAS) is widely recognized as a valuable tool for identifying variants associated with complex phenotypes; the multifactorial nature of NCDs necessitates a more comprehensive exploration, encompassing not only the genetic but also the epigenetic aspect. For this purpose, we employed a bioinformatics-multiomics approach to examine the genetic and epigenetic characteristics of NCDs (i.e. colorectal cancer, coronary atherosclerosis, squamous cell lung cancer, psoriasis, type 2 diabetes, and multiple sclerosis), aiming to identify novel biomarkers for diagnosis and prognosis. Leveraging GWAS summary statistics, we pinpointed Single Nucleotide Polymorphisms (SNPs) independently associated with each NCD. Subsequently, we identified genes linked to cell cycle, inflammation and oxidative stress mechanisms, revealing shared genes across multiple diseases, suggesting common functional pathways. From an epigenetic perspective, we identified microRNAs (miRNAs) with regulatory functions targeting these genes of interest. Our findings underscore critical genetic pathways implicated in these diseases. In colorectal cancer, the dysregulation of the "Cytokine Signaling in Immune System" pathway, involving LAMA5 and SMAD7, regulated by *Hsa-miR-21-5p*, *Hsa-miR-103a-3p*, and *Hsa-miR-195-5p*, emerged as pivotal. In coronary atherosclerosis, the pathway associated with "binding of TCF/LEF:CTNNB1 to target gene promoters" displayed noteworthy implications, with the MYC factor controlled by *Hsa-miR-16-5p* as a potential regulatory factor. Squamous cell lung carcinoma analysis revealed significant pathways such as "PTK6 promotes HIF1A stabilization," regulated by *Hsa-let-7b-5p*. In psoriasis, the "Endosomal/Vacuolar pathway," involving HLA-C and *Hsa-miR-148a-3p* and *Hsa-miR-148b-3p*, was identified as crucial. Type 2 Diabetes implicated the "Regulation of TP53 Expression" pathway, controlled by *Hsa-miR-106a-5p* and *Hsa-miR-106b-5p*. In conclusion, our study elucidates the genetic framework and molecular mechanisms underlying NCDs, offering crucial insights into potential genetic/epigenetic biomarkers for diagnosis and prognosis. The specificity of pathways and related miRNAs in different pathologies highlights promising candidates for further clinical validation, with the potential to advance personalized treatments and alleviate the global burden of NCDs.

1. Introduction

Non-Communicable Diseases (NCDs), such as cardiovascular diseases, cancer, diabetes and neurological disorders, are typically medical conditions that last for extended periods and progress slowly without being contagious [1]. They are responsible for a reduced quality of life and the patients are more susceptible to infectious diseases, due to weakened immune systems. Both the United Nations and the World Health Organization (WHO) predict that by 2030 NCDs will be the leading causes of death globally, surpassing historically important infectious diseases such as cholera, malaria and tuberculosis [2,3]. In addition, these chronic illnesses are increasingly becoming a substantial financial burden on healthcare systems worldwide. Although NCDs are more prevalent in developed countries, they are also a growing concern

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in low- and middle-income countries, where nearly 75% of global NCDs-related deaths have occurred in recent years [1,4]. Since these diseases are characterized by complex and multifactorial aetiologies, involving genetic, environmental and lifestyle factors, addressing the challenge of NCDs requires comprehensive and sustained efforts from the researchers. For this purpose, several computational approaches based, for instance, on machine learning and deep learning have been developed in recent years, to handle the miming and analysis of big data generated by high-throughput techniques in biology and imaging modalities in medicine. These techniques have proved particularly useful, being able to analyze RNA-RNA or protein-protein interactions [5,6].

Similarly, an approach known as Genome-wide association study (GWAS) was developed to identify genetic risk factors for complex diseases. Indeed, through the analysis of the entire genome of large populations, GWAS can identify single nucleotide polymorphisms (SNPs) associated with disease susceptibility [7]. However, the genetic architecture of NCDs is often complex, involving multiple genes, pathways and environmental factors. Therefore, the identification of informative variants and their molecular mechanisms is essential to better understand the disease pathogenesis and thus to obtain reliable biomarkers and to develop effective therapies.

Similarly, another important and emerging tool for the diagnosis and prognosis of complex diseases is the quantification of microRNAs (miRNAs). Indeed, the expression levels of these short non-coding RNA sequences could reflect the patient's health status [8]. However, the current challenge is to identify specific miRNAs as biomarkers for the diseases under investigation [8].

In this study, the complex genetic architecture of the most common or fatal NCDs and the underlying pathogenetic mechanisms have been investigated. In particular, six diseases have been chosen: colorectal cancer (CRC), coronary atherosclerosis (CA), cell lung carcinoma (LC), psoriasis (PSO), Type 2 Diabetes (T2D) and multiple sclerosis (MS). Indeed, these diseases collectively represent a significant global health burden, contributing significantly to morbidity and mortality rates worldwide. Moreover, they encompass a diverse range of non-communicable diseases, thereby enabling us to investigate distinct conditions and mechanisms, seeking specific miRNAs and pathways to be proposed as diagnostic biomarkers. Focusing on these six diseases, we sought specific and reliable biomarkers that could offer valuable insights into genetic mechanisms, that underlie these six distinct, but clinically significant diseases.

Through a computational approach, informative SNPs associated with disease susceptibility for each specific disorder were successfully identified. This was achieved through a linkage disequilibrium clump analysis. Furthermore, the functional implications of these variants were investigated by analyzing their effects on gene expression, transcription factor binding sites and miRNAs regulation. Afterward, in order to gain a comprehensive understanding of the pathological mechanisms, disrupted molecular pathways were explored; redundant miRNAs were identified that are specifically linked to each disease, as new potential biomarkers. The results of our study provide new insights into the complex genetic architecture underlying NCDs and present promising new biomarkers that could be used for the diagnosis and prognosis of NCDs.

2. Materials and methods

2.1. Workflow

The computing approach of this study consists of five steps that we briefly describe below, in Fig. 1.

Step 1 Data Collection

In this study, six GWASs obtained from the public database GWAS Catalog were selected [9]. The selected datasets have participants of European origin and both-sex cohorts to investigate the complex genetic architecture of NCDs in relation to inflammatory, oxidative stress and cell cycle pathways and thus obtain new informative biomarkers. The studies we referred to cover the following phenotypes (Table 1): for the CA dataset, the summary statistics from the United Kingdom Biobank (UKB), which includes approximately 500,000 participants aged 40–69 at recruitment. The CA summary statistics contain data from 16,041 cases and 440,307 controls and information for more than 11 million SNPs (accession ID: GCST90043957, access on 18 October 2022) [10,11]. In relation to CC, summary statistics including 34,869 cases and 29,

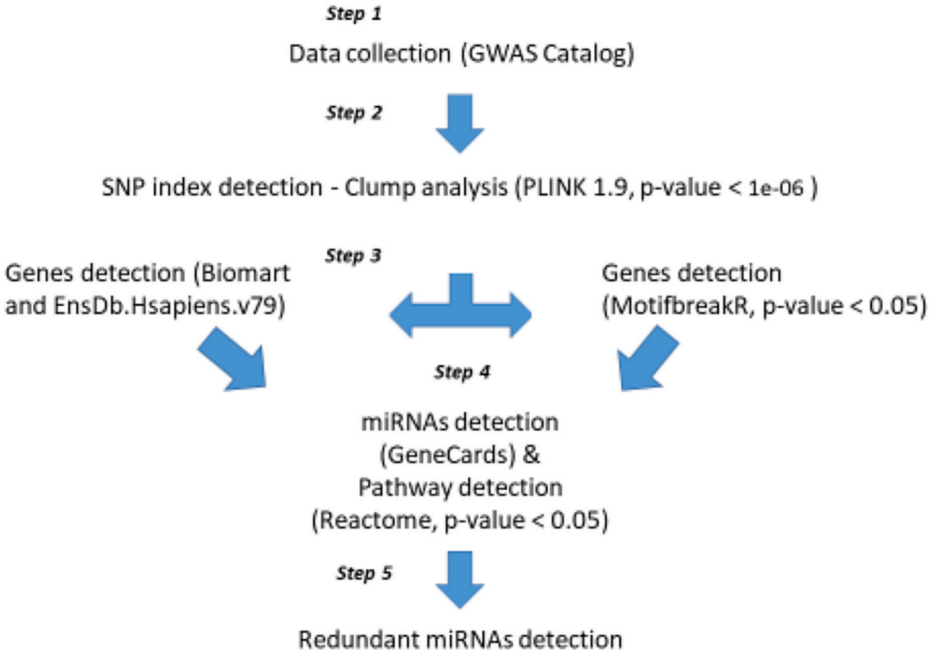

Fig. 1. Flowchart for Non-Communicable Diseases-Related Single Nucleotide Polymorphisms (SNPs), Genes, MicroRNAs, and Pathways Selection: this diagram outlines the systematic approach for selecting SNPs, genes, microRNAs, and pathways associated with Non-Communicable Diseases (NCDs).

Table 1
Summary of Cases and Controls: this table provides an overview of the number of individuals in each category, distinguishing between cases and control subjects.

Pathology	Identification Code	#Cases	#Controls
Coronary Atherosclerosis	GCST90043957	16,041	440,307
Colorectal Cancer	GCST012876	34,869	29,051
Colorectal Cancer (WGS)		1439	720
Colorectal Cancer (Follow-up)		23,262	38,296
Squamous Cell Lung Carcinoma	GCST004750	14,803	12,262
Squamous Cell Lung Carcinoma (Combined Data)		29,266	56,450
Multiple Sclerosis	GCST003566	4888	10,395
Psoriasis	GCST90014456	19,032	286,769
Type 2 Diabetes	GCST90006934	9978	12,348
Type 2 Diabetes (Follow-up)		12,403	16,154

051 controls for GWAS data, and 1439 cases and 720 controls for whole-genome sequencing were considered. Furthermore, an additional 23,262 cases and 38,296 controls were used for follow-up. In a combined meta-analysis of 125,478 individuals, that study identified 40 new independent signals related to CC (accession ID: GCST012876, access on 14 October 2022) [12]. The LC dataset consists of 14,803 cases and 12,262 controls of European ancestry, genotyped on the OncoArray. That study identified 18 susceptibility loci achieving genome-wide significance, including 10 new loci, when combined with existing data for a total of 29,266 cases and 56,450 controls (accession ID: GCST004750, access on 18 October 2022) [13]. For the MS study, the cohort consisted of 4888 cases and 10,395 controls from German participants. That study identified 15 non-MHC loci that reached genome-wide significance in addition to associations within the major histocompatibility complex (MHC) region, with four novel susceptibility loci for MS (accession ID: GCST003566, access on 14 October 2022) [14]. The PSO dataset summary statistics were obtained from UKB and included a cohort of 19,032 cases and 286,769 controls (accession ID: GCST90014456, access on 14 October 2022) [15]. For the T2D study, the GWAS study considered the relation between more than 8.9 million SNPs and T2D risk in 22,326 individuals (9978 cases and 12,348 controls) from the EPIC-InterAct study. A total of 12,403 individuals were identified as incident T2D cases, and a representative sub-cohort of 16,154 individuals was selected from a larger cohort of 340,234 participants for a follow-up time (accession ID: GCST90006934, access on 18 October 2022) [16].

Step 2 Clump analysis

To identify the variants independently associated with the phenotypes of interest, a series of data filtering and analysis steps were performed for each disease. Initially, the most frequent SNPs in the cohorts were selected from the summary statistics from the GWAS, considering only those with a minor allele frequency (MAF) higher than 1% [17]. This is a standard threshold. SNPs with a MAF $<1\%$ are otherwise considered rare and therefore likely to be uninformative [18]. Subsequently, PLINK (version 1.90b) software was applied to compute a linkage disequilibrium clump analysis [19], using the European 1000 Genomes Project as a reference panel [20]. Specifically, since the analyzed phenotypes are considered as multifactorial diseases, our approach creates clusters of variants around SNPs with the lowest p-value in the haplotype, referred to as SNPs index. The aim of this approach was to identify and select the most informative variants that appeared to be independently associated with the traits under examination (i.e., not affected by the linkage disequilibrium), and therefore to remove those variants that were not actually related to the phenotype under investigation [19]. To achieve that, the following threshold values for clumping was established: Clump-p1: 1e-06 (a significant threshold for SNPs index), Clump-r2: 0.001 (a pairwise correlation threshold for LD clumping), and Clump-Kb: 10000 (a physical distance threshold for clumping) (For more information on the parameters, please check: https://www.cog-genomics.org/plink/1.9/postproc#clump.

Step 3 Identification of Altered Genes, TF Binding Sites

After obtaining the lists of SNPs index for each phenotype, two approaches were pursued in parallel to determine which genes were affected by these genetic variants. In the first approach we submitted the lists of SNPs index to two R packages: Biomart (version 2.46.3) [21,22] and EnsDb.Hsapiens (version 79) [23]. These packages allowed us to identify the genes directly affected by our variants. In the second approach, we inputted again the lists of SNPs index to MotifbreakR, (an R package which uses the human hg19 genome as a reference) [24] and thus explored whether variants disrupted transcription factor-binding site motifs (TF-BSM). We applied the "method = ic" provided by the package to estimate the impact of SNPs index on binding motifs, as characterized by HOCOMOCO. This approach employs the relative entropy algorithm to quantify the effects of SNPs on binding motifs [24]. We selected the genes with p-value threshold <0.05. Both approaches were conducted on each phenotype examined in this study.

Step 4 Detection of miRNAs and Disrupted Molecular Pathways

Once the lists of genes affected by our variants were obtained, we submitted them to the GeneCards database, to understand which miRNAs were involved in controlling their expression [25,26] (accessed on 26 October 2022). Furthermore, upon obtaining the lists of genes linked to each disease, we conducted a comprehensive pathway analysis using Reactome software (version 77) (accessed on January 4, 2023) [27]. This analysis identified several pathways that could have been affected by the SNPs index, and consequently by the genes that we previously identified, potentially altering their mechanisms and contributing to disease pathogenesis. To account for multiple testing, those pathways surviving a 5% false discovery rate (FDR) correction (FDR Q value $<$ 0.05) were considered statistically significant. After this correction, we selected those pathways statistically-related to cell cycle mechanisms, inflammation and oxidative stress.

Step 5 Detection of Redundant miRNAs

Finally, pivotal genes within these pathways were successfully retrieved and subsequently the most redundant miRNAs that control their expression, for each disease, were identified. These selected miRNAs emerge as promising candidates, ready to serve as reliable informative biomarkers, offering valuable insights into the complex molecular mechanisms underlying these diseases.

3. Results

In this study a filtering process through MAF selection and clumping analysis was performed in order to identify the most frequent SNPs, that were statistically associated with the diseases under investigation. In the following Table 2, we briefly report the numerosity of SNPs analyzed in each genome-wide association study before and after filters.

These SNPs indexes were further analyzed using Biomart, EnsDb.

Table 2
Summary for SNPs. Numerosity of SNPs analyzed in each genome-wide association study before and after filters.

Pathology	Pre-filtered SNPs	SNPs index
Coronary Atherosclerosis	11,831,932	62
Colorectal Cancer	39,232,598	13
Squamous Cell Lung Carcinoma	7,865,405	16
Multiple Sclerosis	7,968,107	18
Psoriasis	9,419,702	89
Type 2 Diabetes	8,919,079	10

Hsapiens and MotifbreakR R packages to identify the affected genes and the binding site motifs. As expected, the majority of the identified SNPs indexes, detected by the first two packages, were found in intronic regions (80%). Through this approach, a total of 89 CA genes, 19 CC genes, 23 LC genes, 24 MS genes, 98 PSO genes and 15 T2D genes were identified, that could be implicated in pathological processes; subsequently GeneCards software was leveraged to identify the miRNAs that regulate their expression (Supplemental Table 1).

Afterward, the lists of genes for each NCD analyzed were submitted to Reactome software, to identify the pathological pathways associated with cell cycle, inflammatory and oxidative stress. After applying an FDR correction, only statistically significant pathways were selected (Q value < 0.05). Importantly, following this correction, no MS-related pathway appeared to be statistically significant and related to cell cycle, inflammation or oxidative stress. The other NCD pathways obtained from Biomart and EnsDb.Hsapiens genes were mostly related to cell cycle (45%), while inflammation accounted for 33% and oxidative stress 22% of the pathways. The pathways obtained from MotifbreakR genes followed a different pattern, with greater involvement in the cell cycle context (69%), followed by oxidative stress (19%) and inflammation (12%). In some instances, there is evidence of pathway overlap, whereby pathways are involved in multiple processes simultaneously: it is the case of pathways implicated in both cell cycle and oxidative stress processes (e.g., "Oxidative Stress Induced Senescence") (Supplemental Table 2).

Once the pathways statistically correlated with NCDs and related to cell cycle, inflammation and oxidative stress were obtained, the corresponding shared genes between the diseases were investigated, in order to find common pathological molecular profiles. Thus, 9 different genes (*P53*, *MEF2A*, *XBP1*, *MCR*, *RORA*, *TAL1*, *HAL. DQB1*, *ELF3*, *HEY2*) shared between 4 of the NCDs (i.e., CA, PSO, LC, T2D) were identified (Fig. 2).

Afterward, miRNAs linked to genes derived from previous analyses were identified, in particular those associated with cell cycle, inflammation, and oxidative stress for each of the NCDs. Finally, we selected the miRNAs that had the higher number of target genes in relation to each disease (i.e., redundant miRNAs; Supplemental Table 3).

4. Discussion

The present study aimed to investigate the complex genetic architecture of NCDs through a computational approach and thus to identify specific and reliable biomarkers associated with these diseases, within the cell cycle of inflammation and oxidative stress pathways [1–4]. NCDs, including cardiovascular diseases, cancer, diabetes, and neurological disorders, pose a significant burden on global healthcare systems and are predicted to become the leading causes of death worldwide by 2030 [2,3]. These chronic illnesses are characterized by multifactorial aetiologies, involving genetic, epigenetic, environmental and lifestyle factors, making them challenging to understand and treat.

To clarify the genetic basis of NCDs, our study referred to GWAS approach, a powerful tool for identifying genetic risk factors for complex diseases [7] and the role of miRNAs as potential biomarkers for NCDs.

The study successfully identified informative SNPs associated with disease susceptibility for each specific NCD using a linkage disequilibrium clump analysis [7]. Furthermore, the functional implications of these variants were investigated, including their effects on gene expression, transcription factor binding sites and miRNAs regulation. These findings contribute to a better understanding of the genetic architecture underlying NCDs and provide potential targets for the development of effective therapies.

In the next section, we discuss the top 3 significant pathways (based on their p values) meaning cell cycle, inflammation and oxidative stress and their related genes for each disorder. In addition, we contextualize the miRNAs that we identified as possible regulators of the higher number of genes in single pathology among those considered, and in the corresponding pathways. The discussion below does not address MS, which from our analyses did not show statistically significant pathways. This may be due to a limitation of our work caused by to the low statistical resolution of the dataset considered for this study.

4.1. Pathways with a main role in colon cancer

- YAP1- and WWTR1 (TAZ)-stimulated gene expression (cell cycle, p value = 1.85e-06): a previous study showed that dysregulation of the genes *YAP* (Yes-associated protein), *TAZ* (transcriptional co-activator with PDZ binding motif) and *WWTR* (WW domain-containing transcription regulator) could be related to the onset of CC [28]. Specifically, these genes are involved in the Hippo tumor suppression pathway that serves as a central mechanism in the regulation of tissue growth and organ size by limiting cell growth. This pathway is activated when it receives cell cycle arrest signals, including cell polarity, transduction, and DNA damage [29]. Moreover, we confirm a major role for YAP in colon cancer applying the analysis made by TheMarker database (http://themarker.idrblab.cn) [30]. Indeed, it was found as a predictor marker of the clinical outcome after 5-florouracil therapeutic treatment for colon cancer patients.

Our results suggest that *GATA4* (GATA binding protein 4) and *RUNX2* (Runt-related transcription factor 2) are involved in this pathway, as confirmed by the literature [31,32]. In addition, *GATA4* has

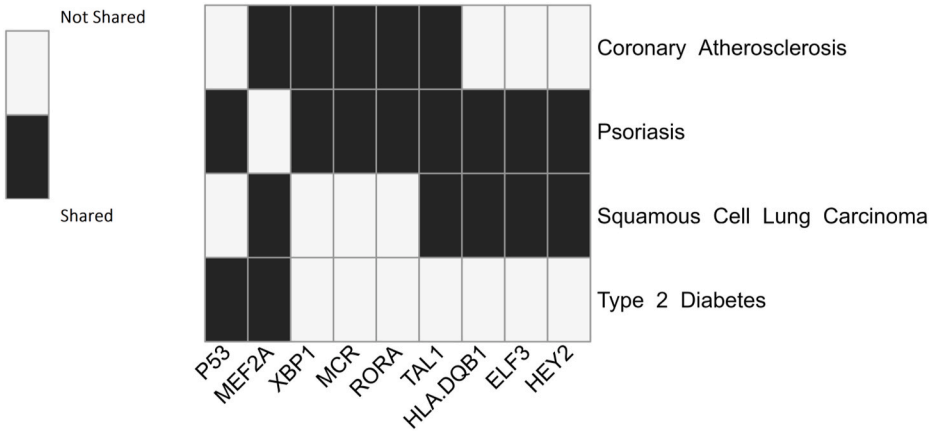

Fig. 2. Shared Genes: the figure displays a list of genes associated with cell cycle, inflammation, and oxidative stress that are shared among the analyzed diseases. Genes highlighted in black are common between two or more phenotypes.

been reported to mediate tumor suppression effects and is often also associated with the proportion of pro-inflammatory macrophages in tumor tissue, suggesting inflammation-driven effects in cancer development [33]. *GATA4* plays a role in enhancing the activity of NFκB, an important inflammatory and tumor-promoting regulator [34]. A further confirm comes from the observation that *GATA4* is expressed in approximately 64% of cancer cases [35]. In addition, this gene is regulated by *Hsa-miR-34c-5p*, an anti-proliferative and pro-apoptotic miRNAs in stage IIIA CC patients [36].

RUNX2 is mainly related to bone development and its tumors [32]. However, a previous study showed that *RUNX2* is implicated in CC; among the other, *RUNX2* is potentially target of the deregulated *Hsa-miR-30d-5p* [37]. Indeed, this miRNA is known to inhibit tumor development in various types of cancer, including CC [37]. This suggests that these genes may play a role in the regulation and the proliferation of CC cells.

The expression of this gene is also controlled by *Hsa-miR-103a-3p*, *Hsa-miR-155-5p*, *Hsa-miR-195-5p*. A previous study showed that *Hsa-miR-103a-3p* is highly expressed in CC cells and that its downregulation inhibits proliferation and promotes apoptosis in CC cell lines, possibly through the TGF-β pathway, suggesting that it could be a potential therapeutic strategy for CC [38]. *Hsa-miR-155-5p* plays a role in CC by regulating chemokine-induced migration of CC cells. A recent study suggests that *Hsa-miR-155-5p* over-expression correlates with poor prognosis in CC patients [39]. The findings suggest that *Hsa-miR-155-5p* acts as a pro-carcinogenic miRNA and may contribute to the increased risk of metastasis in CC patients. Thus, targeting *Hsa-miR-155-5p* may be a potential strategy to inhibit the spread of CC cells to distant sites [39]. Finally, *Hsa-miR-195-5p* is considered a tumor suppressor in CC, as its overexpression suppresses the propagation, invasion and migration of CC cells. Conversely, the transcription level of *Hsa-miR-195-5p* in CC tissues was reduced compared to adjacent normal tissues and it was associated with some clinicopathological factors (lymph node metastasis and tumor grade). Patients with lower *Hsa-miR-195-5p* expression had poorer overall survival compared to those with higher or normal levels, suggesting that *Hsa-miR-195-5p* may serve as a potential prognostic biomarker for CC [40].

- Cytokine Signaling in Immune System (inflammation, p value = 0.0027): cytokine network and inflammation process play a crucial role in the progression of CC at various stages of carcinogenesis [41]. As malignant transformation takes place, both pro-tumorigenic and anti-tumorigenic cytokines become associated. Thus, achieving a delicate balance between pro-inflammatory and anti-inflammatory factors becomes crucial for sustaining homeostasis [41]. Immune cells within the tumor microenvironment act as orchestrators, modulating immune sensitivity and facilitating cancer's escape from immune surveillance [41]. Our findings suggest that *LAMA5* (Laminin alpha5) and *SMAD7* (Mothers against decapentaplegic homolog 7) have an important role in this pathway. Indeed, a recent study demonstrated that increased expression of *LAMA5* is associated with CC angiogenesis processes, responsible for tumor growth and metastasis formation [42]. Furthermore, *LAMA5* expression is regulated by myeloid cells through cytokines signaling, such as TNFα and NF-kB (nuclear factor kappa B), secreted by myeloid cells [42]. *SMAD7* is a gene that codes for an intracellular antagonist of the TGFβ Signaling pathway [43]. It inhibits signal transduction by binding to the TGFβ receptor, leading to the inactivation and degradation of its receptor [44,45]. *SMAD7* expression is upregulated in CC, contributing to TGFβ insensitivity, leading to immune suppression, alterations in cell differentiation, changes in the tumor microenvironment, and promoting tumor progression and metastasis [46–48]. TheMarker database [30] found SMAD7 mRNA expression as a predictor marker of the clinical outcome after 5-florouracil therapeutic treatment.

Both of these genes are regulated by *Hsa-miR-21-5p*, *Hsa-miR-103a-3p* and *Hsa-miR-195-5p* (the last two already discussed). A previous study confirmed that the role of upregulated *Hsa-miR-21-5p* in CC is associated with inducing pyroptosis, a distinct form of programmed cell death characterized by cell swelling and the release of inflammatory factors. Therefore, the findings imply that *Hsa-miR-21-5p* may have therapeutic potential as a target for CC treatment [49].

- Drug-mediated inhibition of CDK4/CDK6 activity (cell cycle/oxidative stress, p value = 0.0035): *CDK4* and *CDK6* are cyclin-dependent kinases with a central role in cell cycle progression: they are involved in the regulation of the G1 phase of the cell cycle by phosphorylating the retinoblastoma protein and allowing progression into S phase [50]. Inhibition of *CDK4* and *CDK6* with drugs like monensin has shown significant benefits in treatment of CC [51]. It is known that oxidative stress can affect cell cycle progression and the activity of cyclin-dependent kinases. Reactive Oxygen Species (ROS) can induce DNA damage, activating cell cycle checkpoints and modulating the activity of various signaling pathways involved in cell cycle regulation. Therefore, it is plausible that oxidative stress may indirectly impact the function of *CDK4* and *CDK6* in CC cells.

Our results suggest that *CCND2* (cyclin D2) might play an important role in this pathway, as it encodes for a cyclin whose function regulates cyclin-dependent kinases [52–54]. In addition, in a previous study a high level of expression of this gene has been observed in CC [55]. The activity of this gene is regulated by regulated by *Hsa-miR-155-5p* and *Hsa-miR-195-5p*, previously discussed.

4.2. Pathways with a main role coronary atherosclerosis disease (CAD)

- SMAD2/SMAD3:SMAD4 heterotrimer regulates transcription (cell cycle, p value = 4.55e-06): by controlling the TGFβ signaling pathway. Indeed, this complex mediates the transcriptional responses to TGFβ by translocating it into the nucleus and regulating the expression of target genes involved in cell growth, differentiation and tumor suppression [56]. Dysregulated TGFβ signaling is a hallmark of many vascular diseases and has been associated with the induction of pathological vascular cell phenotypes, fibrosis and extracellular matrix remodelling [57]. Our study showed that other transcription factors, such as *E2F4 (E2F Transcription Factor 4)*, *MYC* (MYC Proto-Oncogene), BHLH (basic helix-loop-helix) Transcription Factor, *SMAD2* (SMAD Family Member 2), *TGIF1* (TGFβ Induced Factor Homeobox 1) are involved in this pathway. *E2F4* is an inhibitory transcriptional factor downstream of TGFβ [58]. It forms a complex with p107, *E2F5*, *SMAD3* and *SMAD4*, which recognizes and binds to the inhibitory element of the *MYC* promoter, leading to the repression of *MYC* gene expression [59]. This repression contributes to the cytostatic effect of TGFβ [60]. Thus, the dysregulated TGFβ signaling, including the involvement of *E2F4* and *MYC*, can contribute to vascular diseases and pathological vascular cell phenotypes [57].

The activity of this gene is regulated by *Hsa-miR-16-5p* and *Hsa-miR-19a-3p*. An earlier study showed that *Hsa-miR-16-5p* plays a role in regulating multiple processes in CAD, such as programmed cell death and inflammation: its overexpression reduced vascular inflammation and myeloid cell accumulation in the vascular wall, leading to the retardation of plaque formation in mice [61]. In addition, *Hsa-miR-16-5p* is downregulated in the plasma and peripheral blood mononuclear cells of CAD patients, showing to have a protective role in CAD by regulating inflammation and suppressing the plaques formation. Its downregulation could be proposed as a diagnostic biomarker for CAD, and its overexpression or targeted modulation could be a therapeutic strategy for treating CAD [61]. On the other hand, *Hsa-miR-19a-3p* plays a role in the transduction of angiogenic effects in the context of CAD: indeed, this

miRNA is enriched in exosomes released from cells treated with shock wave therapy, a known treatment to induce angiogenesis and to support the regeneration of ischemic hearts, although its angiogenic effects is not fully elucidated and require further investigation [62].

Previous studies demonstrated that *TGIF1* is a transcriptional repressor of TGFβ [63–65], that can be recruited to DNA through two mechanisms: by interaction with SMAD transcription factors, activated by TGFβ, or by repressing transcription independently of TGFβ [66]. *TGIF1* represses the expression of target genes involved in lipid metabolism and cholesterol absorption [66], crucial aspects in the occurrence of CAD. Thus, it is possible that a dysregulation of this gene could lead to the onset of CAD.

Also the expression of this gene is controlled by *Hsa-miR-19a-3p* (previously discussed).

- Binding of TCF/LEF:CTNNB1 to target gene promoters (inflammation, p value = 0.0014): TCF/LEF (T-cell factor/lymphoid enhancer factor) is a family of transcription factors involved in the Wnt Signaling pathway that regulates various biological processes such as embryonic development, cell proliferation, differentiation and inflammation [67]. CTNNB1, also known as β-catenin, is a protein that interacts with TCF/LEF transcription factors and, in the absence of Wnt Signaling, β-catenin is degraded [68]. This prevents TCF/LEF from activating gene expression. When the Wnt Signaling pathway is activated, β-catenin is stabilized and accumulates in the cell, translocates into the nucleus to bind to TCF/LEF transcription factors and this leads to the activation of target genes and various cellular responses [68]. MYC is β-catenin downstream gene target [69] and is activated in atherosclerotic lesions, contributing to the expression of early response genes that propagate atherogenic vascular changes. In addition, it is involved in cell growth, differentiation, and vascular smooth muscle cell proliferation, important processes in atherogenesis [70]. Indeed, activation of MYC in the vascular wall during chronic hypercholesterolemia appears to precede the development of overt atherosclerotic lesions [70]. It is activated in regions of the arterial wall prone to lesion formation and lipid accumulation [71]. MYC is not only regulated by β-catenin, but by NFkB as well [70], a transcription factor involved in atherosclerosis that plays a role in vascular inflammation, cellular proliferation, and apoptosis [72]. Specifically, the p50 subunit of NFkB regulates MYC protein expression by inhibiting its degradation. Therefore, our findings suggest that MYC could play a role in the early stages of atherosclerosis, particularly in lipid accumulation and inflammation.

The activity of this gene is regulated by both *Hsa-miR-16-5p* and *Hsa-miR-19a-3p* (previously discussed).

- Scavenging by Class A Receptors (oxidative stress, p value = 0.0003.): The role of Class A receptors in scavenging is related to their ability to recognize and bind common ligands, including modified forms of low-density lipoprotein (LDL) such as oxidized LDL (OxLDL) [73]. The internalization and degradation of modified LDL particles leads to the formation of foam cells and contributing to chronic conditions like atherosclerosis [74]. Class A receptors (i.e., SR-A1, SR-A3, SR-A4, SR-A5 and SR-A6) are Type II membrane proteins with collagen-like domains and play distinct roles in different tissues and conditions. SR-A1-null mice displayed reduced uptake of acetylated LDL and OxLDL, resulting in decreased atherosclerotic lesions [75]; this suggests that their role is crucial for the onset of CAD.

Our study suggests that APOE (apolipoprotein E), COL4A1 (Collagen Type IV Alpha 1 Chain) and COL4A2 (Collagen Type IV Alpha 2 Chain) have an important role in this pathway. Indeed, APOE exhibits pleiotropic effects that impact various aspects of biological processes, such as plasma lipoprotein metabolism and oxidative processes [76]. Specifically, APOE encodes a protein involved in lipoprotein metabolism and certain variants of that have been implicated in the development of atherosclerosis and cardiovascular disease [77]. APOE plays a role in the clearance of lipoprotein remnants, including OxLDL, which can be recognized and internalized by SR-A receptors [78].

This gene is regulated by *Hsa-miR-16-5p* (previously discussed).

COL4A1 and COL4A2 encode collagen type IV alpha-1 and alpha-2 chains, respectively, which are components of type IV collagen, a major structural protein in basement membranes [79,80], which are crucial for maintaining the integrity and functionality of the basement membrane, particularly in the blood vessel wall [81–87]. Mutations in these genes and consequently disorder of type IV collagen has been implicated in various physiological and pathological processes, including coronary artery disease [88,89]. Indeed, abnormalities in vascular endothelial cells and smooth muscle cells are known to contribute to the development of atherosclerosis [90]. COL4A2 is regulated by *Hsa-miR-16-5p* (previously discussed).

4.3. Pathways with a main role in lung cancer (squamous cell lung carcinoma)

- NOTCH1 Intracellular Domain Regulates Transcription (cell cycle, p value = 1.42e-08): *NOTCH1* (Neurogenic locus notch homolog protein 1) signaling controls cell proliferation in small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) cells [91]. Literature showed that *NOTCH1* pathway has a central role in cancer development [92] and specifically in LC, where it has a tumor inhibitory function, promoting apoptosis [93–95]. Our findings suggest that HES1 (Hairy Enhancer of Split-1), HEY2 (Hairy/enhancer-of-split related with YRPW motif protein 2) and HIF1a (Hypoxia Inducible Factor 1 Subunit Alpha) could play an important role in this pathway. Indeed, HES1 is regulated by NOTCH1 [96] and, being a transcription factor, it is implicated in the regulation of ASH1 (achaete-scute homolog-1) expression in SCLC with neuroendocrine properties [97,98]. In SCLC, ASH1 is degraded by the action of NOTCH1, causing the arrest of cell cycle in G1 phase [99]. A previous study showed that constitutive expression of ASH1 fosters the proliferation of airway epithelial cells, leading to the emergence of pulmonary hyperplasia and metaplasia [100].

The activity of this gene is controlled by *Hsa-let-7b-5p*, which is known to be upregulated during NSCLC tumorigenesis. Indeed, aberrant expression of this miRNA may lead to increased cell proliferation and tumor metastasis. A previous study, aimed to investigate the expression levels of *Hsa-let-7b-5p* in NSCLC tumor samples, showed that it is upregulated in NSCLC compared with non-tumor tissues, indicating a potential oncogenic role in tumor development. The study also proposed the use of *Hsa-let-7b-5p* expression as a diagnostic biomarker of NSCLC [101].

On the other hand, HEY2 is another NOTCH1 target [102]. However, to date we have been unable to identify its role in relation to LC in the literature. HIF1α is a transcription factor, induced by hypoxia status, that plays a crucial role in various biological processes, including tumour cell survival, angiogenesis, invasion [102–104]. It is highly expressed in the tumour microenvironment of LC [105] characterized by high levels of hypoxia. The secretion of certain factors, such as CCL5 (chemokine ligand 5), by HIF1α-expressing fibroblasts has been implicated in promoting tumour growth [105].

The activity of this gene is also controlled by *Hsa-let-7b-5p* (previously discussed).

- Transcriptional regulation of granulopoiesis (inflammation, p value = 0.0043): Granulopoiesis, an essential process within hematopoiesis, culminates in the creation of granulocytes, also known as polymorphonuclear leukocytes. These cells possess nuclei with multiple lobes, typically three, and contain numerous cytoplasmic granules.

This process occurs in the bone marrow and results in the development of three distinct types of mature granulocytes: neutrophils, the most prevalent variety constituting up to 60% of all white blood cells and the main protagonists of the early stages of inflammation [106], followed by eosinophils (up to 4%) and basophils (up to 1%) [46, 107], both present in the sites of allergic inflammations [108].

Our analysis suggests that *RARA* (Retinoic Acid Receptor Alpha) and *TAL1* (TAL BHLH Transcription Factor 1) could play an important role in the LC onset, in relation of this pathway and literature supports this hypothesis. Indeed, it reports that *RARA* is involved in granulopoiesis [109]; since it is also a retinoid receptor, and retinoid has an antitumor effect in LC by modulating angiogenesis and metastasis formation [110], it is possible that aberration of this receptor could sustain LC.

TAL1 likewise appears to be involved in granulopoiesis [111] and since it moreover promotes the TGFβ signaling pathway, it is a central node of a transcriptional regulatory network in LC [112].

- PTK6 promotes HIF1A stabilization (oxidative stress, p value = 0.0123):

PTK6 (Protein Tyrosine Kinase 6) is a cytoplasmic non-receptor tyrosine kinase highly expressed in several tumors, including LC [113]. *PTK6* expression is restricted to differentiated skin and intestinal epithelium in healthy tissues. However, upregulation of that gene is observed in tumors, which is associated with advanced tumor grade, increased tumor size, metastatic potential, and ultimately worse prognosis [114,115].

Our results show that HIF1α is involved in this pathway. As previously discussed, HIF1α is involved in hypoxia processes in LC [105] and hypoxia plays a critical role within the tumor microenvironment, strictly linked to cell proliferation, angiogenesis, metabolism and the tumor immune response. These elements collectively drive tumor advancement, heighten aggressiveness, boost metastatic capabilities and ultimately contribute to adverse prognoses [116]. A previous study related to triple-negative breast cancer showed that PTK6 is recruited and activated by a long non-coding RNA (called LINK-A), in hypoxic environment. Once activated, PTK6 phosphorylates HIF1α, leading to HIF1α stabilization and activation of HIF1α target genes, controlling in triple-negative breast progression and metabolic reprogramming [117]. The expression of PTK6 mRNA is controlled by *Hsa-let-7b-5p* (previously discussed).

4.4. Pathways with a main role in psoriasis

- Interferon gamma signaling (cell cycle and Inflammation, p value = 1.11e-16): Interferon gamma (IFN-γ) is a central pro-inflammatory cytokine in many biological processes [118]. It plays a significant role in the pathogenesis of PSO: it is involved in various mechanisms that contribute to the development and persistence of the disease, such as the stimulation of the proliferation of keratinocytes, thus promoting their excessive reproduction and contributing to the hyper-proliferative nature of psoriatic lesions [119]. Moreover, it stimulates the interaction between inflammatory T cells and keratinocytes, promoting T-cell migration to the lesioned epidermis [120]. It induces the expression of *ICAM-1* (intercellular adhesion molecule 1) and *HLA-DRB1* (Major Histocompatibility Complex, Class II, DR Beta 1), mediating the interactions between T cells and keratinocytes [119]. This interaction leads to the release of several cytokines, such as interleukin IL-1, IL-6, IL-8, TNFα [119]. Previous studies showed that the serum and epidermis concentration of IFN-γ is elevated in patients with PSO compared to healthy individuals [120–122].

This gene is regulated by *Hsa-miR-204-5p*, *Hsa-miR-211-5p*, *Hsa-miR-4755-5p*, *Hsa-miR-5006-3p*, *Hsa-miR-623* and *Hsa-miR-6832-3p*. *Hsa-miR-204-5p* is related to immunity and inflammation: its specific role in PSO is unclear, but a previous study suggests that triptolide, a compound extracted from a Chinese medicinal herb, may exert its anti-inflammatory effects and alleviate psoriatic symptoms through the upregulation of the expression of *Hsa-miR-204-5p*. Furthermore, researchers showed that triptolide inhibits Th17 cell response (known to be involved in the development of PSO) through *Hsa-miR-204-5p*-mediated suppression of gene target phosphorylation [123].

In addition, our study showed the involvement of the genes *HLA-C* (Major Histocompatibility Complex, Class I, C), *HLA-DQA1* (Major Histocompatibility Complex, Class II, DQ Alpha 1), *HLA-DQB1*, *HLA-DRB1* (previously discussed) and *HLA-DRB5* (Major Histocompatibility Complex, Class II, DR Beta 5) in this pathway. *HLA-C* in PSO is as a risk allele within the region on 6p21, specifically in the PSORS1 region. *HLA-Cw6*, also referred to as HLA-C*06:02 has consistently shown the strongest association with PSO risk [124]. However, the effects of the genetic architecture of the MHC region on the risk of OSP are not fully understood.

The activity of this gene is regulated by *Hsa-miR-148a-3p*, *Hsa-miR-148b-3p*, *Hsa-miR-152-3p* and *Hsa-miR-6854-5p*. A previous study investigated the role of *Hsa-miR-148a* and *Hsa-miR-148b* as two immune-related miRNAs in patients with autoimmune skin diseases, specifically PSO and vitiligo. The study aimed to assess the expression profile of *Hsa-miR-148a/b*, correlating it with the clinical features of the diseases. The expression levels of both miRNAs were found to be elevated in PSO patients compared to healthy controls. In addition, both miRNAs showed to have a role in regulating various physiological processes in the skin, such as keratinocyte proliferation, differentiation as well as immune regulation [125]. However, to date we have been unable to find information in the literature regarding miRNAs *Hsa-miR-152-3p* and *Hsa-miR-6854-5p* in relation to this disease.

HLA-DQA1 and *HLA-DQB1* have been implicated as risk variants for PSO. Polymorphisms in these genes, particularly amino acid positions within the HLA antigens' binding sites, demonstrate stronger associations with PSO risk than classical HLA alleles [124]. Finally, on the role of *HLA-DRB5* in PSO, nothing is reported specifically in the literature so far. This gene is regulated by *Hsa-miR-211-5p*, *Hsa-miR-4755-5p*, *Hsa-miR-5006-3p*, *Hsa-miR-623* and *Hsa-miR-6832-3p*. To date, we have not found any information in the literature that specifies its role concerning PSO.

- Endosomal/Vacuolar pathway (oxidative stress, p value = 1.11e-16): The endosomal pathway plays a crucial role in antigen presentation and cross-presentation [126]. Usually, the antigen-containing endosome fuses with a lysosome and degrades the antigen into smaller amino acids by the action of ROS. This allows class II MHCs to be loaded with extracellular antigens. However, changes in pH as a result of ROS imbalances can trigger the pathway known as "cross-presentation". The result is the processing and presentation of exogenous antigens to class I MHC molecules [127]. Class I MHCs have many functions ranging from the identification of viral or tumor peptides to the identification of "self" (i.e., antigens normally present in tissues) [128,129]. Knowing that PSO is an autoimmune disease and seeing that our results suggest an involvement of the *HLA-C* gene (regulated by *Hsa-miR-148a-3p*, *Hsa-miR-148b-3p*, *Hsa-miR-152-3p* and *Hsa-miR-6854-5p,* previously discussed), it is possible that an imbalance in this mechanism could lead to misrecognition as "non-self" of normally "self" antigens and thus to the onset of PSO.

4.5. Pathways with a main role in type 2 diabetes

- The pathway statistically most associated with inflammation in this disorder is "Binding of TCF/LEF:CTNNB1 to target gene promoters" (p value = 0.0034). However, since we have already examined this pathway in relation to CA, we will discuss the second pathway statistically most associated with inflammation.
- Regulation of TP53 Expression (cell cycle, p value = 5.36e-06):

Our analyses suggest that *TP53* (Tumor Protein 53) and its regulation could play an important role in T2D context. TP53 is a tumor suppressor protein that is known to regulate the cell cycle by inducing cell cycle arrest, senescence and apoptosis [130]. Specifically, the activation of *TP53* leads to pause the progression of the cell cycle and thus it allows DNA repair or, if the damage is irreparable, triggering apoptosis. Additionally, *TP53* has been found to regulate *mTOR* (the mammalian Target of Rapamycin), a key regulator of protein synthesis, cell growth, proliferation and autophagy [131]. The negative regulation of *mTOR* by *TP53* occurs through the transactivation of negative regulators of *mTOR*, such as *AMPK* (5′ AMP-activated protein kinase), *TSC2* (tuberous sclerosis complex protein 2), and *PTEN* (phosphatase and tensin homolog) [131,132]. These regulators inhibit *mTOR* activity, leading to growth cessation and induction of autophagy. Therefore, *TP53* acts as a negative regulator of *mTOR*, influencing cell cycle progression and cellular metabolism [133].

In relation to T2D, a prior investigation unveiled a correlation between genetic variations in the TP53 pathway and the likelihood of developing diabetes. Notably, a particular variation known as the codon 72 polymorphism of TP53 showcases contrasting capabilities in triggering cell cycle arrest and apoptosis between its P72 and R72 variants. The R72 variant has been associated with a higher risk for T2D and increased insulin resistance [133]. Furthermore, a previous study using mouse models for the codon 72 polymorphism of *TP53* demonstrated that mice with the R72 variant developed more severe obesity, glucose intolerance, insulin resistance, and fatty liver disease compared to mice with the P72 variant when fed a high-fat diet [133]. This suggests that the dysregulation of *TP53* can influence metabolic phenotypes and thus lead to the T2D onset.

The activity of *TP53* is controlled by the following miRNAs: *Hsa-miR-106a-5p*, *Hsa-miR-106b-5p*, *Hsa-miR-155-3p*, *Hsa-miR-17-5p*, *Hsa-miR-19b-3p*, *Hsa-miR-20a-5p*, *Hsa-miR-2110*, *Hsa-miR-26a-1-3p*, *Hsa-miR-324-5p*, *Hsa-miR-4271*, *Hsa-miR-454-3p*, *Hsa-miR-4651*, *Hsa-miR-4725-3p*, *Hsa-miR-5193*, *Hsa-miR-608* and *Hsa-miR-6780b-5p*. A previous study explored the effects of hypoglycemia on the expression of some miRNAs, in patients with T2D. The study found that following a hypoglycemic episode, the levels of *Hsa-miR-106a-5p* were increased at both 1- and 7-days post-hypoglycemia [134]. Similarly, the expression levels of *Hsa-miR-106b-5p* were also increased. On the other hand, *Hsa-miR-17-5p*, *Hsa-miR-20a-5p*, *Hsa-miR-26a-1-3p* and *Hsa-miR-324-5p* were altered and downregulated from hypoglycemia at 4 h and 24 h in the control group [134]. Another study reported that *Hsa-miR-155-3p* is downregulated in serum levels of T2D patients. In terms of the molecular mechanisms, this miRNA is involved in the regulation of various biological processes, including immune response, hematopoiesis and inflammation; in T2D, its downregulation may contribute to pancreatic beta-cell dysfunction and insulin resistance. In adipose tissue and skeletal muscle, *Hsa-miR-155* downregulation may be associated with insulin resistance. Thus, *Hsa-miR-155* downregulation in T2D patients may serve as a potential biomarker for the disease and could be targeted for therapeutic interventions [135].

Another study showed that *Hsa-miR-19b-3p* is downregulated in obese mice with diabetic cardiomyopathy induced by a high-fat diet. This miRNA is involved in the alteration of glucose and lipid metabolism via insulin pathways and has been proposed as biomarkers for diabetes prognosis in clinical trials [136].

On the other hand, *Hsa-miR-454-3p* was identified as one of the differentially expressed miRNAs in the serum of patients with T2DM compared to healthy subjects. Indeed, a previous study showed that *Hsa-miR-454-3p* is upregulated in patients with T2DM. *Hsa-miR-454-3p* may play a role in regulating insulin sensitivity, insulin resistance, glucose homeostasis and lipid metabolism. The specific role of *Hsa-miR-454-3p* in the pathogenesis of T2DM is not fully elucidated however it could be useful as potential biomarker for the disease [137].

Another study showed that *Hsa-miR-5193* may be involved in the pathogenesis and progression of T2D-associated peripheral neuropathy. The mechanism is still unclear, however it may play a role in lipid metabolism, TGF-β receptor signaling pathway, lipid transport and the *PPAR* (peroxisome proliferator-activated receptor) signaling pathway. These pathways are known to be involved in the development and progression of T2D-associated peripheral neuropathy [138].

Recent research showed that *Hsa-miR-608* appears to be related to T2DM. Specifically, it is mentioned that Hsa-circRNA11783-2 sponges *Hsa-miR-608*, sequestering or inhibiting its activity. By binding to *Hsa-miR-608*, *Hsa-circRNA11783-2* might prevent *Hsa-miR-608* from interacting with its target genes, thereby regulating their expression. This interaction suggests that *Hsa-circRNA11783-2* may have a regulatory role in T2DM through its ability to modulate *Hsa-miR-608* activity [139]. Instead, we have been unable to find information in the literature to date regarding this disease and miRNAs *Hsa-miR-2110*, *Hsa-miR-4271*, *Hsa-miR-4651*, *Hsa-miR-4725-3p* and *Hsa-miR-6780b-5p*.

- TRAF6 mediated induction of NF-kB and MAP kinases upon TLR7/8 or 9 activation (inflammation, p value = 0.004): *TRAF6* (TNF Receptor Associated Factor 6) regulates the activation of NF-kB through the phosphorylation of *IKK* (Inhibitor of Nuclear Factor Kappa B Kinase Subunit Beta) complex by *TAK1* and the *MAPK* (Mitogen-activated protein kinases) cascades [140,141]. Subsequently, the activation of these genes leads to the initiation of immune response and thus the production of pro-inflammatory cytokines and antimicrobial peptides [142,143].

Our study showed an involvement of *MEF2A* (Myocyte Enhancer Factor 2A, controlled by *Hsa-miR-19a-3p* and *Hsa-miR-19b-3p*, previously discussed) and *P53* (controlled by *Hsa-miR-106a-5p*, *Hsa-miR-106b-5p*, *Hsa-miR-155-3p*, *Hsa-miR-17-5p*, *Hsa-miR-19b-3p*, *Hsa-miR-20a-5p*, *Hsa-miR-2110*, *Hsa-miR-26a-1-3p*, *Hsa-miR-324-5p*, *Hsa-miR-4271*, *Hsa-miR-454-3p*, *Hsa-miR-4651*, *Hsa-miR-4725-3p*, *Hsa-miR-5193*, *Hsa-miR-608* and *Hsa-miR-6780b-5p*, previously discussed) in this pathway. *MEF2A* is a transcription factor involved in many activities related to growth factor, stress-induced pathways, myocardial development [144–146] and regulation of the expression of inflammatory factors, such as *MCP-1* [147]. on the role of *MEF2A* in T2D, nothing is reported specifically in the literature so far.

- Formation of Senescence-Associated Heterochromatin Foci (SAHF) (Cell Cycle/Oxidative Stress, p value = 0.014): The process of DNA damage/telomere stress-induced senescence, triggered also by oxidative stress, leads to the formation of senescence-associated heterochromatin foci (SAHF). Specifically, senescent cells exhibit SAHF, distinct regions of facultative heterochromatin dedicated to repressing genes that promote proliferation [148]. Each SAHF consists of a condensed chromosome, with telomeric and centromeric chromatin located primarily at its periphery [149–151]. The formation of SAHF involves the activity of an evolutionarily conserved protein complex composed of HIRA and ASF1a. As cells approach senescence, this complex accumulates at the PML bodies, which are nuclear structures that contain various proteins, including the PML protein, and are involved in assembling regulatory complexes and modifying proteins. The localization of HIRA to PML bodies is suggested to play a catalytic role in the formation of SAHF. As cells become senescent, HIRA and HP1 proteins transiently colocalize in PML bodies before being deposited in SAHF, indicating a dynamic process in SAHF formation [152–154].

Our analyses suggest an involvement of *TP53* (regulated by *Hsa-miR-106a-5p*, *Hsa-miR-106b-5p*, *Hsa-miR-155-3p*, *Hsa-miR-17-5p*, *Hsa-miR-19b-3p*, *Hsa-miR-20a-5p*, *Hsa-miR-2110*, *Hsa-miR-26a-1-3p*, *Hsa-miR-324-5p*, *Hsa-miR-4271*, *Hsa-miR-454-3p*, *Hsa-miR-4651*, *Hsa-miR-4725-3p*, *Hsa-miR-5193*, *Hsa-miR-608* and *Hsa-miR-6780b-5p*, already discussed) in this pathway. Particularly, the formation of SAHF itself also relies on functional *TP53* pathways [155]. Cellular senescence is

characterized by cellular inability to divide, to remain metabolically active, and to release a wide range of pro-inflammatory cytokines, chemokines, and growth factors; this phenotype is collectively known as senescence-associated secretory phenotype. It has emerged as potentially responsible for the pathogenesis and progression of T2D and its associated complications [156]. The accumulation of senescent cells within adipose tissue, a prevalent phenomenon in obesity, can induce adipose tissue dysfunction, inflammation and impaired responsiveness to insulin [156]. Furthermore, cellular senescence has been linked to pancreatic β-cell dysfunction and reduced insulin secretion, both of which are fundamental characteristics of T2D [157,158]. Addressing cellular senescence by either eliminating senescent cells (senolytic therapy) or modulating the SASP holds promise as a therapeutic approach for treating T2D and its complications [156].

Interestingly, T2D showed more redundant miRNAs than other NCDs. The causes of this difference require further study, but may suggest a more prominent role of epigenetics in this disease. Alternatively, it is possible that the phenotypic spectrum of T2D is broader than in other NCDs, affecting several organs, and thus its molecular mechanisms may be related to multiple pathways. This phenotype could lead to the alteration of several miRNAs in this pathology. Finally, given the high incidence of T2D compared with the other diseases examined [159,160], it is possible that this one has been characterized further and therefore more data on it are available.

5. Conclusions

In conclusion, the present study emphasizes the importance of pathways related to cell cycle, inflammation and oxidative stress, which have been found to play a crucial role in the development and progression of NCDs. The complex genetic and epigenetic architecture of these disorders involves multiple genes, pathways and environmental interactions, making them difficult to understand and treat. Indeed, the use of tools such as GWAS alone are not adequate to describe in detail all the molecular processes underlying non-Mendelian diseases [161]. Our study used a multiomics approach starting with GWAS to identify variants as possible specific and reliable biomarkers associated with these diseases in the context of cell cycle, inflammation, and oxidative stress. Indeed, the analysis revealed informative SNPs associated with disease susceptibility and functional implications of these variants. Through an examination of the pathways and associated genes affected by these SNPs in each disorder, we have elucidated the molecular mechanisms of these diseases. This involves identifying genes that have a specific impact on the disorders treated and identifying those that are shared. Such insights deepen our understanding of the biological basis of these disorders, opening avenues for progress in diagnosis, prevention, and personalized medicine. Indeed, our findings are confirmed by the literature, but also suggest the involvement of new genes of interest, yet unexamined in depth, as possible targets for further study. In addition, we explored the role of miRNAs as potential biomarkers and clinical target, although identification of specific miRNAs remains difficult because of their ability to regulate the expression of different genes. While our study does not take into account the environmental component, our results contribute to a better understand the genetic architecture and molecular mechanisms underlying these disorders. They provide valuable insights into potential therapeutic targets and thus the development of effective treatments. Further research is needed to validate and explore the functional implications of these biomarkers in the clinical setting.

Ethical approval

This study was conducted using summary association data generated by previous studies. Owing to the use of previously collected, deidentified and aggregated data, this study did not require institutional review board approval.

CRediT authorship contribution statement

Salvatore D'Antona: Conceptualization, Data curation, Formal analysis. **Danilo Porro:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Francesca Gallivanone:** Conceptualization, Supervision, Writing – review & editing. **Gloria Bertoli:** Data curation, Methodology, Supervision, Writing – original draft.

Declaration of competing interest

The authors reported no biomedical financial interests or potential conflicts of interest.

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Appendix A. Supplementary data

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