Environmental pollution and COVID-19: the molecular terms and predominant disease outcomes of their sweetheart agreement

Inquinamento ambientale e COVID-19: le basi molecolari della loro interazione

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ABSTRACT

As the Coronavirus situation (COVID-19) continues to evolve, many questions concerning the factors relating to the diffusion and severity of the disease remain unanswered.
Whilst opinions regarding the weight of evidence for these risk factors, and the studies published so far are often inconclusive or offer contrasting results, the role of comorbidities in the risk of serious adverse outcomes in patients affected with COVID-19 appears to be evident since the outset. Hypertension, diabetes, and obesity are under discussion as important factors affecting the severity of disease. Air pollution has been considered to play a role in the diffusion of the virus, in the propagation of the contagion, in the severity of symptoms, and in the poor prognosis. Accumulating evidence supports the hypothesis that environmental particulate matter (PM) can trigger inflammatory responses at molecular, cellular, and organ levels, sustaining respiratory, cardiovascular, and dysmetabolic diseases.
To better understand the intricate relationships among pre-existing conditions, PM, and viral infection, we examined the response at the molecular level of T47D human breast adenocarcinoma cells exposed to different fractions of PM. T47D cells express several receptors, including the aryl hydrocarbon receptor (AhR), and ACE2, the main – but not the only – receptor for SARS-CoV-2 entry.
PM samples were collected in an urban background site located in the Northern area of the City of Bologna (Emilia-Romagna Region, Northern Italy) during winter 2013. T47D cells were exposed to organic or aqueous (inorganic) extracts at the final concentration of 8 m^3 for a 4-hour duration. Both the concentration and the exposure time were chosen to resemble an average outdoor exposure. RNA was extracted from cells, purified and hybridised on 66k microarray slides from Agilent.
The lists of differentially expressed genes in PM organic extracts were evaluated by using Metacore, and an enrichment analysis was performed to identify pathways maps, process networks, and disease by biomarkers altered after T47D treatment.
The analysis of the modulated genes gave evidence for the involvement of PM in dysmetabolic diseases, including diabetes and obesity, and hypertension through the activation of the aryl hydrocarbon receptor (AhR) canonical pathway.
On the basis of current knowledge, existing data, and exploratory experimental evidence, we tease out the likely molecular interplay that can ultimately tip the disease outcome into severity. Looking beyond ACE2, several additional key markers are identified. Disruption of these targets worsens pre-existing conditions and/or exacerbates the adverse effects induced by SARS-CoV-2 infection. Whilst appropriately designed, epidemiological studies are very much needed to investigate these associations based on our hypothesis of investigation, by reviewing recent experimental and epidemiological evidence, here we speculate and provide new insights on the possible role of environmental pollution in the exacerbation of effects by SARS-CoV-2 and other respiratory viruses. This work is intended to assist in the development of appropriate investigative approaches to protect public health.

Keywords: COVID-19, particulate matter, SARS-CoV-2, inflammation, diabetes, hypertension, obesity

WHAT IS ALREADY KNOWN

- **Chronic and acute exposure to high concentration of air particulate matter (PM) can exacerbate existing heart conditions and play a role in the development of heart and cerebrovascular disease, through the promotion of a state of inflammation.**
- **PM is also recognized as an endocrine disruptor playing a role in the development of dysmetabolic diseases, such as diabetes and obesity, which are risk factors for hypertension and cardiovascular disease.**
- **Diabetes, obesity, and hypertension have been described as important factors affecting the severity and prognosis of COVID-19.**

WHAT THIS PAPER ADDS

- **The paper reports and discusses the role of key receptors and other transmembrane proteins in the complex response to a concomitant exposure to PM components and virus which may share the same molecular mechanisms and affect the same molecular targets.**
- **The analysis of molecular signatures in cells exposed to PM highlights the molecular mechanisms sustaining key events possibly involved in the development of diabetes, obesity, and hypertension, providing a mechanistic interpretation of epidemiological evidence.**
- **The comparison of PM-induced key events with those induced by SARS-CoV-2, through the disruption of the same molecular targets, strongly suggests plausible interplays at the molecular level, in which case the additive exposure to PM could be regarded as another risk factor for developing severe forms of COVID-19.**

RIASSUNTO

Con il progredire dell'epidemia di COVID-19, non è ancora possibile fornire una risposta certa al quesito su quali siano i fattori in grado di influenzare la diffusione del virus e la gravità della malattia.
Sebbene le opinioni a riguardo divergano e gli studi presentino spesso risultati non conclusivi o contrastanti, è apparso evidente sin dall'inizio il ruolo delle patologie pregresse e concomitanti, tra cui ipertensione, diabete e obesità, nel sostenere un aumento del rischio di manifestazioni più severe in pazienti COVID-19. Anche l'inquinamento è stato preso in considerazione come un possibile facilitatore della diffusione del virus e della propagazione del contagio e come fattore importante nell'esacerbazione dei sintomi e nella prognosi infausta.
È ben noto, d'altronde, che il particolato atmosferico (PM) induce una risposta infiammatoria, rilevabile a livello molecolare, cellulare e d'organo, associata alla possibile evoluzione di malattie respiratorie, cardiovascolari e dismetaboliche.
Per meglio comprendere la complessa interazione tra patologie preesistenti, PM e infezione virale, è stata esaminata la risposta molecolare all'esposizione a diverse frazioni di PM, utilizzando la linea cellulare di adenocarcinoma mammario umano T47D. Queste cellule esprimono diversi recettori, tra cui il recettore aril-idrocarburo (AhR), molecola chiave per la risposta biologica agli inquinanti ambientali, e ACE2, il principale – ma non unico – recettore utilizzato da SARS-CoV-2 per penetrare nella cellula.
I campioni di PM sono stati raccolti nell'area Nord di Bologna durante l'inverno 2013. Le cellule sono state esposte per 4 ore a estratti organici o acquosi di PM alla concentrazione finale di 8 m^3. Dalle cellule è stato estratto l'RNA, poi purificato e ibridizzato su vetrini microarray Agilent di 66k.
La lista dei geni modulati dall'esposizione a estratti organici è stata analizzata con il software Metacore, con una *enrichment analysis* per identificare i *pathway* genici, i processi e i biomarcatori di malattia correlati alla modulazione. Questa analisi ha permesso di rilevare il ruolo del PM nei processi correlati all'ipertensione e alle malattie dismetaboliche, tra cui diabete e obesità, mediante l'interazione con AhR e la modulazione della risposta biologica correlata a questo recettore.
Sulla base delle attuali conoscenze, corroborate dalla letteratura scientifica, e delle evidenze sperimentali ottenute, è stata ipotizzata un'interazione a livello molecolare che potrebbe spiegare un possibile ruolo del PM nell'esacerbazione delle manifestazioni cliniche conseguenti all'infezione virale. Oltre al rettore ACE2, sono state identificate altre molecole chiave, la cui modulazione potrebbe portare a un peggioramento delle condizioni cliniche preesistenti o degli effetti conseguenti l'infezione.
Sebbene siano necessari studi epidemiologici appropriati per verificare questa ipotesi sperimentale, il presente studio, condotto nell'ottica di stimolare nuovi approcci investigativi a supporto degli interventi di sanità pubblica, offre una diversa chiave di lettura sul ruolo che l'esposizione ambientale può avere nell'evoluzione dell'infezione sostenuta da SARS-CoV-2 e, più in generale, dai virus respiratori.

Parole chiave: COVID-19, particolato, SARS-CoV-2, infiammazione, diabete, ipertensione, obesità

INTRODUCTION

As the Coronavirus outbreak (COVID-19) continues to evolve, with the number of cases still dramatically rising in many countries, several questions about the factors affecting the diffusion and severity of the disease remain unanswered.
The new Coronavirus, SARS-CoV-2 was named due to its ability to induce severe acute respiratory syndrome and its phylogenetic link with its sister clade, SARS-CoV.[1] However, as the time goes by, the baffling nature of SARS-CoV-2 is becoming more and more evident, due to the unpredictability of the virus behaviour and the plethora of induced symptoms, of which the respiratory syndrome is only the most known. The clinical spectrum ranges from asymptomatic disease to moderate symptoms, including fever, coughing and loss of smell and taste, which may resolve in few weeks. to severe pneumonia and death due to multiple organ failure. In addition, moderate to severe coagulation disorders have frequently been reported.[2,3]
Therefore, it is of paramount importance to identify the factors that can affect the diffusion of the virus and the severity of the disease and to better understand which groups of population are at higher risk to develop the most severe symptoms, so that they can be better protected.
The list of potential risk factors that have been recognized to date includes male gender, age over 65, smoking habit and pre-existing medical conditions.[4] Additionally, the way in which the role of environmental pollution may be an additional risk factor for severe illness is also currently being hotly debated.[3,5-7]
Whilst, as yet, there may not yet be a comprehensively developed general opinion regarding the weight of evidence for these risk factors, and, for now, available studies are often inconclusive or offer contrasting results, the role of comorbidities in the risk of serious adverse outcomes in patients affected with COVID-19 has been clear since the outset. On admission, 20%-50% of COVID-19 patients have been reported to have at least one comorbidity, with hypertension, diabetes, cardiovascular, and cerebrovascular diseases being amongst the most common.[2,8] Indeed, the association with medical conditions including hypertension, diabetes, and obesity, appears to be evident.[9]
Hypertension affects 26% of the population worldwide, particularly from middle age onwards, and with a predominance in males.[10]
Hypertension has been reported in almost 30% of hospitalized COVID+ (COVID-19-test positive patients) patients and it is associated with 2.5-fold higher risk of severe COVID-19.[11,12] It is still unclear whether uncontrolled blood pressure is a risk for acquiring COVID-19 or whether patients taking medications to control hypertension are at a lesser risk.[12] It is also debated as to wheth-

er hypertension is a risk factor for developing the most severe forms of COVID-19, or if the Coronavirus disease is responsible for severe hypertension in COVID+ patients.[11] The link between COVID-19 and hypertension is not entirely surprising since the virus efficiently binds with the angiotensin-converting enzyme 2 receptor (ACE2), a member of the renin-angiotensin-bradykinin system (RAS), also known as the renin-angiotensin-aldosteron system (RAAS), which regulates body fluid homeostasis and blood pressure, although ACE2 axis-mediated regulation of blood pressure is only one of the pathways through which this complex balance is ensured. The high prevalence of diabetes is also considered an important comorbidity in patients with COVID-19. Diabetes was recognized as a comorbidity in 10-20 % of COVID+ patients hospitalized in China and in 33% of the COVID+ patients admitted to 12 hospitals in New York City area during the first month of the outbreak.[8,13] This is not unexpected, as it is well known that individuals suffering from diabetes are at higher risk for respiratory infections, including influenza and pneumonia.[14] Furthermore, diabetes has previously been reported as a risk factor for mortality in infected patients from H1N1 (2009 swine flu), SARS, and MERS.[15] MERS Coronavirus (MERS-CoV) showed an even more active role in diabetes, because it uses the receptor-binding domain of human dipeptidyl peptidase IV (DPP4) as the gateway for entry into human cells. DPP4 is a ubiquitous, multifunctional glycoprotein playing multiple roles in nutrition, metabolism, the endocrine system, bone marrow mobilization, cancer growth, and cell adhesion, and as such is a target for type-2 diabetes therapy.[16]

Accumulating evidence also suggests that obesity increases risk of severe COVID-19.

A recent report by *Public Health England* reviewing and analysing information from 12 UK studies and 19 international studies[17] focused on the relationship between excess weight and COVID-19. The report concluded that obesity could dramatically increase cases and worsen the prognosis arising from COVID-19 infection. The impact of the conclusions of this meta-analysis immediately raised the governmental impetus to improve policy options to reduce the incidence of obesity in the UK.

To date, nationally, the UK has higher overweight rates than Italy (63% versus 40%, when considering BMI>25).[17,18] But Italy is not lagging far behind, and childhood obesity particularly is becoming a relevant and growing problem in Italy.

In Italy, 99% of deaths from COVID-19 have been in patients with pre-existing conditions, including those which are commonly seen in people with obesity, such as hypertension, cancer, diabetes, and heart diseases.

The Italian national health institute report on 4,942 deaths, provided useful information about comorbidities involved in COVID-19 deaths (Istat-Iss: 2020). Mean age at death from COVID-19 is around 80 years. Obesity is associated with deaths in COVID-19 patients for less than 10%, a percentage not higher than that observed in general population of the same age. However, when considering younger deaths (up to 59 years, almost 6% of total deaths), cancer and obesity became the most frequent cofactors (22% and 17%, respectively for 50-59 years). Obesity is the major cause of deaths up to 49 years (20% of 64 deaths).

As with many pathologies, obesity appears more frequently in association with other causes of death rather than being the only contributing cause. This consequence is not unexpected, as obesity is not a lethal condition, but it does predispose and thus contribute to a greater vulnerability of individuals to infectious diseases such as COVID-19.

Obesity is correlated with age, sex, and ethnicity. An association with soicoeconomic status, is often reported. Indeed, some of the studies analysed in the UK report17 suggest that the association between COVID-19 and obesity is attenuated by, but independent of, socioeconomic factors.

Life-style is considered to play the main role in the increasing incidence of obesity, as the consequence of relatively high dietary intakes of sugar, fast food, and trans fatty acids[19,20] large portion sizes, and decreased physical activity.[21]

Environmental pollution has also been considered to play a role in the diffusion of the virus, in the propagation of the contagion, in the severity of symptoms and in the poor prognosis.[3 5-7]

Environmental particulate matter (PM) is a complex mixture containing inorganic and organic chemicals of toxicological concern. We have previously described the pathway-based toxicity elicited by PM in in vitro models and highlighted the molecular mechanisms leading to several adverse outcomes through the activation of the aryl hydrocarbon receptor (AhR).[3,22-24]

The complex AhR-ARNT binds specific DNA sequences, triggering the AhR signalling pathway, which leads to the transcription of the CYP1A1 and CYP1B1 genes, whose proteins play the main role in the bioactivation of several xenobiotics.[25] We have previously speculated that PM and some of its chemical components may contribute to exacerbate the symptoms in COVID+ patients through the modulation of the same molecular pathways, leading to the amplification of the signals sustaining the inflammatory response.[3,5] The phenomenon of hypercytokinemia, commonly known as the cytokine storm, with the overproduction of early response cytokines, such as tumour necrosis factor (TNF), interleukin-6 (IL-6), and interleukin-1β (IL-1β), characterizes the most severe forms of COVID-19, and it is associated with an increased risk of vascular permeability, multiorgan failure, and death.[3,26]

A low-grade inflammatory process, characterized by the release of inflammatory cytokines, occurs in both diabetes and hypertension.[27]

Furthermore, there is a substantial overlap between the mechanisms by which diabetes; hypertension and PM can contribute to boost the inflammatory response in COVID+ patients. This overlap highlights the intriguing hypothesis of a molecular interplay, whereby ACE2 definitely plays a central role, but where the final outcome is cross talk teamwork sustained by multiple receptors and cognate ligands.
In the subsequent discussion of this speculative work we will explore such molecular interplay, utilizing current knowledge, existing data, and exploratory experimental evidence.

STRUCTURE AND INFECTIVITY OF SARS-COV-2 AND THE ROLE OF ECTOPEPTIDASES IN THE VIRUS-HOST INTERACTION

SARS-CoV-2 is an enveloped positive strand RNA virus. The envelope is derived from the host cell, with the typical structure of cell membranes (phospholipids and proteins), and glycoproteins of viral origin, forming spikes on the surface of the envelope. The spike glycoprotein (S) is responsible for the host tropism and engages with the host receptor to enable host cell entry. It is the seventh human Coronavirus of zoonotic origin that has been identified so far.[28] All human Coronaviruses induce respiratory infections, from the common cold (HCoV-OC43 and HCoV-229E), to mild respiratory infections, such as bronchiolitis (HCoV-NL63 and HCoV-HKU1) to severe respiratory syndromes (SARS-CoV, MERS-CoV, and SARS-CoV-2). Surprisingly, all Coronaviruses use ectopeptidases as entry receptors.[29-33] An overview of the Coronavirus receptors is reported in supplementary material.
Three Coronaviruses, HCoV-NL63, SARS-CoV, and SARS-CoV2, bind with the ectopeptidase ACE2, but with different consequences. Whilst the binding of SARS-CoV and SARS-CoV-2 with ACE2 leads to an abnormal and adverse inflammatory response, the binding of ACE2 with HCoV-NL63 rarely induces similar severe effects.[29] Indeed, binding to the receptor is a determinant for the host tropism, but it is not sufficient to ensure either the completion of the virus entry process, which is supported by accessory proteins, or to support the virus infectivity and the severity of the diseases, which is linked to host factors. These host factors include the efficiency of the immune response together with the host ability to counteract the viral stratagems, to escape such a response. Both the virus and the host develop their own strategies through evolutionary genomic modifications in a race to win what is known as the "host-virus arms race".[34] Most of the receptors show polymorphisms conferring higher or lower affinity to bind with the virus. Viruses undergo random mutations with consequent viral evolution that shapes the viral infectivity.[35] Several receptors, particularly ACE2, work within a network of proteins, each of which performs a specified receptor-cognate task as part of a multifaceted communication network that is trying to maintain a homeostatic balance by dealing with a homeostatic threat.[36] Different variants of the same virus can cooperate to boost their own reproductive chances, by enabling multiple viral genomes to be co-transmitted within the same cell.[37]
As we start to appreciate the complexity of the interaction of the virus with the human organism, the contributory factors to consider increase. It has been reported that genetic variations in both the host ACE2 sequences and the viral spike proteins are one of the main barriers to the virus diffusion across the species as well as their contribution to the susceptibility and/or resistance against the viral infection.[38] Therefore, the probability to be infected, the course of the disease, the severity of the symptoms, and fatal outcomes may be the combined consequence of ACE2 variants and comorbidities. Even more interestingly, it has been speculated that the binding of SARS-CoV-2 and ACE2 is not unique and requires the cooperation of the ACE2 network proteins. The network includes 6 membrane-bound proteins, ACE2, DPP4, MEP1B, MEP1A, MME, PRCP, and XPNPEP2.[39-48] A brief description of the functions of these proteins is reported in supplementary material.
DPP4 and MEP1A were found to bind with eight proteins of SARS-CoV-2, with the exception of protein S.[36] The involvement of DPP4 is further confirmed by the recognized high binding affinity of two DPP4 inhibitors used in diabetic patients, saxagliptin, and sitagliptin, which has been used pharmaceutically against all three SARS-CoV-2 surface proteins.[36] MEP1A is a metalloprotease that has been described as cooperating with disintegrins ADAM10 and ADAM17 to generate the soluble form of IL-6, the pro-inflammatory cytokine.[43] This cytokine plays the master role in triggering the abnormal inflammatory response.[43] We have recently suggested that ADAM17 is the bridging molecule in the interplay of PM and SARS-CoV-2 leading to the cytokine storm and may likely be responsible for the exacerbation of the inflammatory response described in COVID+ patients. This preliminary and as yet unconfirmed evidence, together with the data discussed herein gives rise to a key question: is the exposure to PM the third co-morbidity in COVID-19, together with hypertension and diabetes?

HYPERTENSION, DIABETES, OBESITY AND PM: TEAM (INTER) PLAYERS IN THE COVID-19 SCHEMA

It is well known that the exposure to PM is related to cardiovascular effects. Associations are consistent across the epidemiological studies and supported by evidence from the analysis of molecular markers in individuals exposed to PM, which provides the biological plausibility.[49-52]
While it is well known that health conditions related to chronic inflammation, such as diabetes, coronary artery disease and past myocardial infarctions, confer higher susceptibility to air pollution-related cardiovascular effects,

much less is known about the effects elicited by short-term exposures related to acute inflammatory response.[53] Some literature reports give evidence for blood pressure alterations after short-term exposure to increased levels of $PM_{2.5}$ and suggest that long-term exposure may be linked to chronic hypertension.[54]

Within an ongoing EU funded research project exploring the effects of Severe Air Pollution Episodes (SAPEs) in three functional urban areas in Italy, Poland, and Hungary, we have suggested that individuals affected by hypertension and/or diabetes may be more sensitive to the detrimental effects of acute episodes of air pollution.[55] These adverse effects could be even more severe in aging citizens.[55] Indeed, episodes of acute air pollution exposure (both via outdoor and indoor routes) have been reported to be associated with raised pulse rates and blood pressure, both of which are predictive markers for adverse cardiovascular events in elderly people taking antihypertensive medications.[56] High blood pressure in diabetic patients can foster diabetic complications such as coronary artery disease. It has been reported that diabetic patients exposed to increased levels of PM, nitric dioxide, and sulphur dioxide are at risk of higher blood pressure.[57] This is probably due to the ability of PM and other air pollutants to trigger an inflammatory response.[57] Surprisingly, the risk is higher in diabetic patients at younger age and at normal weight.[57]

Several environmental pollutants have been postulated to be "obesogens" for their ability to disrupt energy metabolism[58-60] and type 2 diabetes[61] and are being investigated further.[62] Obesity has been described as a susceptibility factor for the effects of the indoor PM on cardiovascular disease[63] and key genes in inflammatory pathways have been reported to be methylated in obese individuals exposed to PM_{10}.[64]

We have demonstrated before that the exposure to PM and its components in in vitro models modulates gene pathways involved in the immune-mediated inflammation, sustaining an adverse pathway that leads to increased vascular permeability and coagulation disorders as well as diabetes and pregnancy adverse outcomes.[3,22-24]

Our previous results give strong evidence for a molecular interplay of receptors, their physiological substrates and their exogenous ligands, which could lead to the exacerbation of the inflammatory response in COVID+ patients related to the main severe outcomes like the pulmonary intravascular coagulopathy (PIC), which is associated with the immune-response and considered an immune-mediated fibrosis.[3]

To better understand the intricate relationships among pre-existing conditions, PM, and viral infection, we examined the response at the molecular level of T47D human breast adenocarcinoma cells exposed to different fractions of PM. The reason for the selection of these cells was due to their expression of a functional aryl hydrocarbon receptor (AhR), plus several other hormone receptors, including steroid hormone receptors (oestrogen receptors, the androgen receptor, progesterone receptor, glucocorticoid receptor, prolactin receptor, and the growth hormone receptor), and several key receptors of RAAS, including both angiotensin type 1 receptor, AT1 and AT2 mitochondrial assembly receptor (MAS) and ACE2.[65-67] Whilst it is acknowledged that these cells do not express other receptors of the ACE2 network, and therefore are not representative of lung as the main target organ for both PM and the new Coronavirus, they still represent a suitable model to explore the interaction of PM and its components with molecular targets critical in SARS-CoV-2 entry and replication.

EFFECTS OF PM ON GENE PATHWAYS RELATED TO INFLAMMATION-DEPENDENT DIABETES AND GLUCOSE METABOLISM DISORDERS: EXPERIMENTAL EVIDENCE

MATERIALS AND METHODS

$PM_{2.5}$ and PM_1 were sampled in an urban background site located in the northern area of the city of Bologna during winter in 2013. T47D cells were exposed to organic and aqueous (inorganic) extracts of $PM_{2.5}$ or PM_1 fractions at the final concentration of 8 m^3. The exposure was carried out for 4 hours. Both the concentration and the exposure time were chosen to resemble an average outdoor exposure. Four replicates for each treatment were carried out, individually managed from RNA extraction to labelling and hybridization. The lists of differentially expressed genes in $PM_{2.5}$ and/or PM_1 organic extracts from Main Site were evaluated by using Metacore, an integrated knowledge-based platform with a manually annotated database of protein interactions verified by small experiment data and gene-disease associations. In particular, an enrichment analysis was performed to identify Pathways Maps, Process Networks, and Disease by Biomarkers altered after T47D treatment. Experimental details are reported in supplementary material.

RESULTS

Organic fractions from both $PM_{2.5}$ and PM_1 were able to modulate gene pathways involved in diabetic diseases (table 1).

The list of the genes with the highest modulation is reported in table 2.

The genes listed in table 2 are all included in gene pathways and involved in biological processes sustaining metabolic disorders and leading to diabetes and obesity. Whilst hypertension is not listed among the diseases supported by these processes, it is important to note that several of the modulated genes identified in table 2, specifically genes involved in nitric oxide regulation, such as thromospondin-1 and endothelin-1, are also involved in the regulation of blood pressure, confirming that hypertension represents one of the main complications in both diabetes and obesity.

DISEASE	FDR	
	$PM_{2.5}$	PM_1
Diabetes Mellitus	1.074E-02	4.528E-05
Glucose Metabolism Disorders	1.910E-03	1.931E-04
Diabetes Mellitus, Type 2	1.431E-03	2.212E-04
Body Size	1.131E-04	1.882E-03
Metabolic Diseases	1.560E-03	4.528E-05
Body Weight	1.131E-04	1.980E-03
Overweight	1.131E-04	6.136E-03
Obesity	1.792E-04	5.648E-03
Overnutrition	1.792E-04	5.648E-03
Nutritional and Metabolic Diseases	2.154E-04	2.777E-04
Nutrition Disorders	5.550E-04	2.169E-03

FDR: False Discovery Rate
Data generated using Metacore. / Dati generati con Metacore.

Table 1. Metabolic and nutritional disorders correlated to gene pathways modulated by organic extracts of PM2.5, PM1 (FDR<0.05).
Tabella 1. Modulazioni relative a disordini della nutrizione, del metabolismo del glucosio e diabete indotte dall'estratto organico di PM2.5, PM1 (FDR<0,05).

To better understand this relation and highlight molecular signatures common to metabolic disorders and cardiovascular effects, we analysed the genes involved in cardiovascular disease and that were dysregulated after the exposure of cells to PM. The list of these genes is reported in table 3. Most of the modulated genes reported in table 3 are involved in the regulation of epithelial secretion of electrolytes. During the embryogenesis process, these genes control vasculogenesis and heart development. In adult life, the complex network regulated by HES1 and HEY1, which includes ID1 and ID2, plays a role in macrophage activation and cardiovascular calcification. Thrombospondin-1 is the only gene involved in both metabolic and cardiovascular disorders.

It should be noted that as this data is based upon 4 replicates, using only one cell line, these exploratory results require further confirmation utilizing other in vitro cell models, particularly the front-line lung epithelial cells directly exposed to PM. Work underway, and not reported here, is exploring the molecular pathways, genes, and responses to assess whether there are constitutive differences between the T47D carcinoma cell-line, as compared with lung cell lines and non-carcinomatous cell lines.

DISCUSSION

CYP1A1 AND THE CENTRAL ROLE OF ARYL HYDROCARBON RECEPTOR (AHR) IN MEDIATING PM EFFECTS

AhR is a highly evolutionarily conserved environmental sensor, known to bind dioxins and polycyclic aromatic hydrocarbons, but also several endogenous ligands, including tryptophan derivatives. The canonical AhR pathway activates genes of the metabolic pathways, mainly cytochrome P450 1A1 (CYP1A1), to sustain cell detoxification. The expression of CYP1A1 is almost totally dependent on AhR activity.[22,25] Therefore, the strong up-regulation of CYP1A1 confirms the PM directed activation of the AhR and the AhR pathway in this cell model. As described previously, binding to the AhR represents the molecular initiating event in the cascade of signalling pathways and molecular key events leading to adverse outcomes related to the exposure to PM.[3,22-24] We have also reported that the AhR can interplay with other receptors in T47D cells directly or indirectly through the cell microenvironment,[68] sustaining inflammation and inflammation-related disorders that are described in COVID+ patients.[3]

The analysis of genes dysregulated by the exposure of T47D cells to PM gives exploratory evidence for the intense cross-talk among gene pathways that are common to obesity, diabetes, hypertension, and other related complications. Whilst the data here needs to be confirmed in further relevant in vitro models, there is supporting clinical evidence in the scientific literature, with respect to the role of the AhR in hyperglycaemia and vascular complications in diabetic patients, through the formation of a complex with several transcription factors activated by glucose.[69] To provide an integral picture of this complex interrelationship and in the view of the recent epidemiological evidence regarding obesity as one of the main risk in COVID-19 fatality,[17] we considered the most significant genes, arranged in two functional groups: genes involved in adipogenesis and dysregulated metabolism, and genes coding for substrates or ligands of the two receptors, DDP4 and meprins, involved in SARS-CoV-2 entry. The role of dysregulated genes in hypertension, as a complication of both diabetes and obesity, is also discussed.

Gene interplay, pathway crosstalk, and gene involvement in SARS-CoV-2 diffusion and contagion as well as in severity and fatality of COVID-19 is discussed further here.

OBESITY: THE EPIDEMIC WITHIN THE NEW PANDEMIC. DOES THE MOLECULAR INTERPLAY SUPPORTED BY PM EXPOSURE EXPLAIN AN ADDITIONAL RISK FOR COVID-19?

Diabetes is a common comorbidity with obesity. Insulin-resistance and type 2 diabetes are considered obesity-related complications.

At the nuclear receptor mediated molecular level, metabolism, and obesity disrupting effects are considered to be mediated via the peroxisome proliferator activated recep-

GENE SYMBOL	GENE NAME	FOLD CHANGE	UP/DOWN REGULATION
ADRA2A	Alpha-2 A adrenergic receptor	1.555	UP
C/EBP zeta	CCAAT/enhancer binding protein, zeta	1.771	UP
CCL2	Monocyte Chemotactic Protein 1	1.554	UP
CCR7	C-C Motif Chemokine Receptor 7	1.852	UP
CHRM	Cholinergic Receptor Muscarinic 1	3.201	UP
CXCR4	C-X-C chemokine receptor type 4	-1. 681	DOWN
CRF o CRH	Corticotropin releasing hormone	3.210	UP
CYP1A1	Cytochrome P450 1 A1	101.620	UP
EDN1	Endothelin-1	1.297	UP
FAM65C	RIPOR family member 3	1.715	UP
GAD1	Glutamate decarboxylase 1	8.030	UP
GLUT1	Glucose transporter 1	1.711	UP
GLUT4	Glucose transporter 4	-1.507	DOWN
HGF receptor	Hepatocyte Growth Factor	-1.680	DOWN
HMOX1	Heme oxigenase 1	2.109	UP
HSP70	Heat shock protein70	2.029	UP
HSPA1A	Heat shock protein A	2.021	UP
HSPA1B	Heat shock protein B	2.021	UP
IL15RA	Interleukin-15 receptor subunit alpha	1.501	UP
IL34	Interleukin-34	1.559	UP
iNOS	Inducible nitric oxidase synthase	-1.894	DOWN
Integrin	Integrin	-2.220	DOWN
IP3 receptor	Inositol 1,4,5-trisphosphate receptors	3.659	UP
ITGB2	Integrin Subunit Beta 2	-2.220	DOWN
LIPIN1	Lipin-1	-1.693	DOWN
MGF	Mast cell growth factor	-2.133	DOWN
MOG	Myelin oligodendrocyte glycoprotein	1.992	UP
NRP1	Neuropilin-1	2.072	UP
PARD6	Partitioning defective 6 homolog alpha	-1.629	DOWN
PDF	Peptide deformylase	1.768	UP
PERC	PPARG coactivator 1 beta	1.722	UP
Resistin	Resistin	1.812	UP
S1P2 receptor	Sphingosine-1-phosphate receptor 2	1.538	UP
SDF-1	Stromal Cell-Derived Factor 1	-2,161	DOWN
SHISA9	Shisa family member 9	-3.093	DOWN
sIL-15RA	Interleukin 15 Receptor Subunit Alpha	1.501	UP
SOC53	Suppressor of cytokine signaling 3	-1.578	DOWN
TRF1	Telomeric repeat-binding factor 1	-3.439	DOWN
THBS1	Trombospondin – 1	-1,498	DOWN
TWEAK	TNF-related weak inducer of apoptosis	1.698	UP
VDR	Vitamin D receptor	1.559	UP
VIP receptor	Vasoactive intestinal polypeptide receptor	2.262	UP
WNT	Wnt family member	-2.056	DOWN

Table 2. Genes modulated in T47D cells after the exposure to PM and associated with metabolic and nutritional disorders.
Tabella 2. Geni modulati in cellule T47D esposte a PM e associati a disordini metabolici e nutrizionali.

GENE SYMBOL	GENE NAME	FOLD CHANGE	UP/DOWN REGULATION
GATA4	GATA binding protein 4	-1.436	DOWN
HES1	hes family bHLH transcription factor 1	2.478	UUP
HEY1	hes related family bHLH transcription factor with YRPW motif 1	-2.400	DOWN
HOPX	HOP homeobox	-2.051	DOWN
ID1	inhibitor of DNA binding 1, HLH protein	-1.675	DOWN
ID2	inhibitor of DNA binding 2	-1.42	DOWN
ITPR1	inositol 1,4,5-trisphosphate receptor type 1	3.624	UP
TBX2	T-box transcription factor 2	-1.43	DOWN
THBS1	thrombospondin 1	-1.574	DOWN
PKD1	polycystin 1, transient receptor potential channel interacting	1.260	UP
WNT3A	Wnt family member 10B	1.40	UP
ZIC3	Zic family member 3	-1.54	DOWN

Table 3. Genes modulated in T47D cells after the exposure to PM and associated with cardiovascular disease.
Tabella 3. Geni modulati in cellule T47D esposte a PM e associati alla patologia cardiovascolare.

tors (PPARs), particularly PPARα and PPARγ, but several other receptors found principally in the liver are also involved in metabolic homeostasis (e.g. PXR, CAR, LXRα, LXRβ, FXR, not discussed here).[70] PPARα oxidizes fatty acids in the liver cells, and PPARγ stimulates storage of fatty acids in the adipose tissues. Glucose transporters are very much related to PPARγ activation, and adverse perturbation is associated with high blood glucose, such that glitazone pharmaceuticals have been developed as PPARγ agonists to lower glucose levels.

GLUT1 and GLUT4 genes, coding for glucose transporter 1 and glucose transporters 4, are respectively up-regulated and down-regulated in our cell model. The down-regulation of GLUT4 in response to the PM exposure is particularly significant, since reduced levels of GLUT4 mRNA have been detected in diabetes patients. GLUT4 plays a key role in insulin-stimulated glucose uptake in adipocytes and muscle. In obesity, type 2 diabetes and insulin-resistance, the expression of GLUT2 remains unchanged in muscle, while it dramatically decreases in adipocytes, showing the importance of the adipocyte tissue in metabolism disorders.[71]

It is also notable that PPARγ coactivator 1 beta (PERC), is identified in our model. PPARγ coactivators are multifunctional transcriptional tissue-specific coregulators active in many metabolic pathways. The expression of PERC, also known as PGC-1β, increases in response to dietary fat intake and leads to hyperlipidemia. The effects of PERC on plasma triglyceride metabolism are regulated by several factors, including the members of the lipins family.[72] Lipin-1 is a bifunctional protein involved in the enzymatic regulation of the triglyceride synthesis. It directly affects insulin sensitivity. Deficiency of lipin-1, population polymorphisms of lipin-1 gene or low levels of lipin-1 in humans are all related to increased insulin resistance.[73,74]

The downregulation of lipin-1 gene (LIPN) in our model confirms the interplay between this gene and PERC in response to obesogenic factors. In addition to a role in obesity, the inactivation of host LIPN has been described as one of the most important RNA viruses' strategies to accelerate viral replication,[75] and this really does begin to explain the key role of this gene in SARS-CoV-2 infection.

Intriguingly, resistin (RETN) also known as adipose tissue-specific secretory factor (ADSF), a gene coding for a hormone linking obesity to type-2 diabetes, by inducing resistance to insulin[76] is upregulated in our experimental T47D model, upon exposure to PM. While resistin is secreted by adipocytes in rodents, under the control of the PPARγ, in humans it is also expressed by peripheral blood mononuclear cells (PBMC) and macrophages, in response to pro-inflammatory cytokines, supporting the role of human resistin in the pathogeneses of inflammation and obesity-related diseases.[77]

Aspects of obesity can be considered to be low-grade inflammatory conditions sustained by the infiltration of macrophages into adipocytes.[78-80] Macrophages derive from the differentiation of monocytes orchestrated by chemokines. IL-34 is a novel interleukin, involved in several signalling pathways and biological functions, including the process of differentiation and polarization of macrophages. Obese individuals have increased levels of circulating IL-34 correlated with insulin-resistance and high expression of IL-34 in adipocytes, which is enhanced by the pro-inflammatory chemokines TNFα and IL-1β.[81] In obese patients IL-34 increases fat accumulation and inhibits the effect of insulin on glucose transport.[81] IL-34 is upregulated in our model, suggesting a mechanism whereby exposure to PM can activate the inflammatory response sustaining obesity and triggering insulin-resistance. The up-regulation of another key gene, CCL2, involved in the recruitment of monocytes, indicates that, in our experimental model at least, the complex immune-mediate inflammatory response to PM components affects key genes involved in obesity.

Our results give also evidence for a possible role of PM in adipogenesis through the modulation of vitamin D receptor (VDR). VDR mediates the inhibitory effects of vitamin D3 on adipogenesis.[82] VDR mRNA levels in adipose tissue are higher in obese individuals, and VDR expression is considered a marker of insulin resistance.[83] VDR regulates more than 200 genes, including those involved in the uptake and transportation of calcium. Thus, it is not surprising that the upregulation of VDR in our model is associated with the strong upregulation of inositol triphosphate (IP3), a gene regulating the calcium influx.

Whilst vitamin D deficiency has been associated with obesity,[79] it is generally a common deficiency particularly over the age of 40 years and in periods of reduced sunlight exposure.[84,85]

In relation to COVID-19 risks, Vitamin D supplementation has been recently recommended (https://www.nhs.uk/conditions/vitamins-and-minerals/vitamin-d/).[79,86,87]

The role of vitamin D in contrasting infections from respiratory viruses, including: syncytial virus, influenza viruses, and Coronaviruses has been reported.[88,89] Mechanistically, the action of vitamin D against virus infection has been attributed to its ability to modulate key molecules of the pathway of immune response. [90] It is also known that there is a role for vitamin D in the regulation of RAS.[91,92] Indeed, vitamin D can downregulate ACE2, by suppressing RAS through the VDR pathway.[91] This evidence, combined with our results, suggests a possible intriguing mechanistic interplay of PM and SARS-CoV-2 along the VDR pathway.

PM exposure in our model leads to the strong down-regulation of the telomeric repeat-binding factor (TRF1). This gene encodes for a protein that is a member of the telomere nucleoprotein complex, and can inhibit telomerase shortening, slowing down the aging process. In our model, TRF1 dysregulation, can be considered to be indicative of the effect of PM upon premature aging.

The aging process is known to be sustained by the shortening of telomeres, the repeated DNA sequences at the ends of chromosomes.[93,94] Individuals affected by diabetes mellitus have shorter telomeres compared to non-diabetics. Telomere shortening observed in both type 1 and type 2 diabetes may be responsible for the premature organ aging, which is a well-known consequence of diabetes.[95] Some literature reports give evidence for a moderate inverse association between obesity and telomere length.[96] In humans, telomere shortening is directly related to adiposity.[97] This correlation, however, is not linear across the age and it is more evident in young obese patients compared to elderly obese patients.[97]

The maintenance of genome integrity is a fine example of the host-virus arms race, with viruses developing strategies to maintain the host genomic integrity, which is functional to virus replication, and the host attempting to disrupt virus telomeric repeats.[98] Interestingly, telomere shortening has been reported in leucocytes of patients at risk of developing severe and fatal COVID-19. This apparent discrepancy in the SARS-CoV-2 strategy to ensure its own replication is possibly explained by the immune efficiency that telomere length confers to leucocytes to counteract infections. While leucocyte shortening is an aging condition, and the elderly are at higher risk of dying from COVID-19, leucocytes telomeres length is a heritable human trait, showing high variability among individuals. Therefore, all conditions that are characterized by accelerating telomere shortening, including obesity and cancer, facilitate virus infection and predispose to worse outcomes. By dysregulating TRF-1, a gene that plays a key role in maintaining the telomere length, PM may interplay with the virus strategy in accelerating the aging of leucocytes, leading to the severe leukopenia associated with COVID-19 fatal outcomes.

AHR AND THE CROSSTALK WITH ACE2 NETWORK PROTEINS: THE ROLE OF CORONAVIRUS RECEPTOR SUBSTRATES IN THE RESPONSE TO PM EXPOSURE

From the analysis of our results, a key role of genes coding for DPP4 and meprins substrates emerges, although neither DPP4 nor meprins have been reported to be expressed in T47D cells, as yet. Chemokine ligand 2 (CCL2), corticotropin releasing factor (CRF), stromal cell-derived factor 1 (SDF-1), and the ligand of vasoactive intestinal polypeptide receptor (VIP receptor) are all substrates of DPP4,[99,100] while endothelin-1 is a substrate of MEPA.[41] An overview of the function of these genes, of their role and the role of their products in diabetes and its complications and/or in the inflammatory response and/or in hypertension and cardiovascular disease is reported in supplementary materials.

CRF, also known as "corticotropin releasing hormone" (CRH) or "corticoliberin", is a member of the hypothalamus-pituitary-adrenal (HPA) axis, which also includes the adrenocorticotrophic hormone (ACTH) and glucocorticoids (mainly cortisol in humans). It is strongly upregulated in our model. CFR-1 coordinates the stress response to several stress stimuli, through the production of ACTH, which, in turns, modulates the production of glucocorticoids. While the role of CRF in the inflammatory response is supported by several studies, its role in diabetes is controversial.[101] The metabolic stress induced by diabetes and the consequent continuous activation of HPA has been suggested to be a possible cause of insulin-resistance.[102] The HPA axis, including CRF, has been put forward in explaining the stress response to SARS-CoV-2 infection due to an interplay with RAS.[103] This hypothesis is based upon the post mortem evidence of very high concentrations of viral particles detected in the adrenal and the pituitary organs of the patients who died from COVID-19.[103]

CCL2, also known as monocyte chemotactic protein 1 (MCP-1), is a promoter of inflammation, renal injury, and fibrosis in diabetic nephropathy. The inflammation associated with diabetes induces an innate immune response in the kidney through the accumulation of kidney macrophages. This process is fostered by CCL2 through the recruitment of monocytes into the kidney, which, in turns, activate the macrophages. The production of proinflammatory cytokines following the macrophages activation leads to the injury of kidney parenchymal cells and supports the fibroblast proliferation. This can evolve into kidney fibrosis with associated decline of renal function.[104] The upregulation of CCL2 is also responsible for hypertension. Indeed, monocytes/ macrophages, that migrate in response to CCL2, are mediators of hypertensive inflammation. Elevated levels of angiotensin II induce the upregulation of CCL2, increasing, in turn, high levels of circulating CCL2 in hypertensive patients.[105] CCL2 together with chemokine receptor 2 (CCR2) plays an important role in the defence against neurotropic Coronavirus infection by regulating T cell polarization, trafficking, and macrophage accumulation.[106] CCL2 is up-regulated by PM exposure in our model.

The up-regulation of CCR7, another CC chemokine receptor, that has a fundamental role in balancing the immune response against chemical and biological xenobiotics with tolerance towards self-antigens, further highlights the effect of PM on molecular targets integral in both diabetes complications and host defence. The involvement of CCL2 in the response to Coronavirus infection has been largely described in previous reports related to SARS.[107,108] CCL2 has been described as the earliest upregulated chemokine in lung epithelial or monocyte-derived dendritic cells infected with SARS-CoV.[109] Increased plasma levels of CCL2 is both a marker of disease progression in SARS patients and a marker of therapeutic intervention in patients treated with corticosteroids to reduce hypercytokinemia.[108,110] CCL2 functional polymorphisms have been reported to increase the susceptibility to Coronavirus infection.[108] The

overexpression of CCL2 sustains the migration of monocytes and macrophages as well as their infiltration in lung tissues, related to SARS, MERS, and SARS-CoV-2.[111,112] This accumulated evidence provides a legitimate basis for a key role of CCL2 in the switch from innate to aberrant immune response to infection, sustained by the Coronavirus. Analogously, the upregulation of CCL2 in response to PM exposure in our cell model marks the step from adaptive to maladaptive cell response.

SDF-1, also known as C-X-C motif chemokine 12 (CXCL12), functions as a chemotactic cytokine and proangiogenic chemokine, which enhances hematopoietic and endothelial progenitor cell recruitment to sites of cell injury.[112] It is expressed in microvascular endothelial cells within the pancreatic islets and in surrounding interstitial stromal tissue. The role of SDF-1 in diabetes is debated. In experimental studies in rodents, it has been demonstrated to have a role in the mitigation of diabetes and improve the survival of pancreatic beta cells.[113] However, SDF-1 has also been described to worsen the vascular complications in diabetes patients treated with gliptins, the incretin-based inhibitors of DPP4, known as the fourth generation of diabetes therapeutics, which seem to potentiate SDF-1.[114]

In our model, the upregulation of endothelin-1, a potent vasoconstrictor peptide, confirms our previous reports on the role of PM in the microvascular dysfunction leading to the endothelial disease.[3,24] Microvascular dysfunction is the cause of most diabetes complications, including retinopathy, nephropathy, and neuropathy. Diabetic microangiopathy is often characterized by increased levels of endothelin-1.[115] High levels of endothelin-1, have been found in heavy smokers and in individuals suffering hypertension, atherosclerosis, coronary heart disease, cerebrovascular diseases, and sepsis.[116]

Associations between endothelin-1 and exposure to air pollution in elderly persons, among males but not females, have been previously reported, suggesting that the increase in plasma endothelin-1 together with the increase in air pollution may be one of the pathway leading to changes in blood pressure.[117]

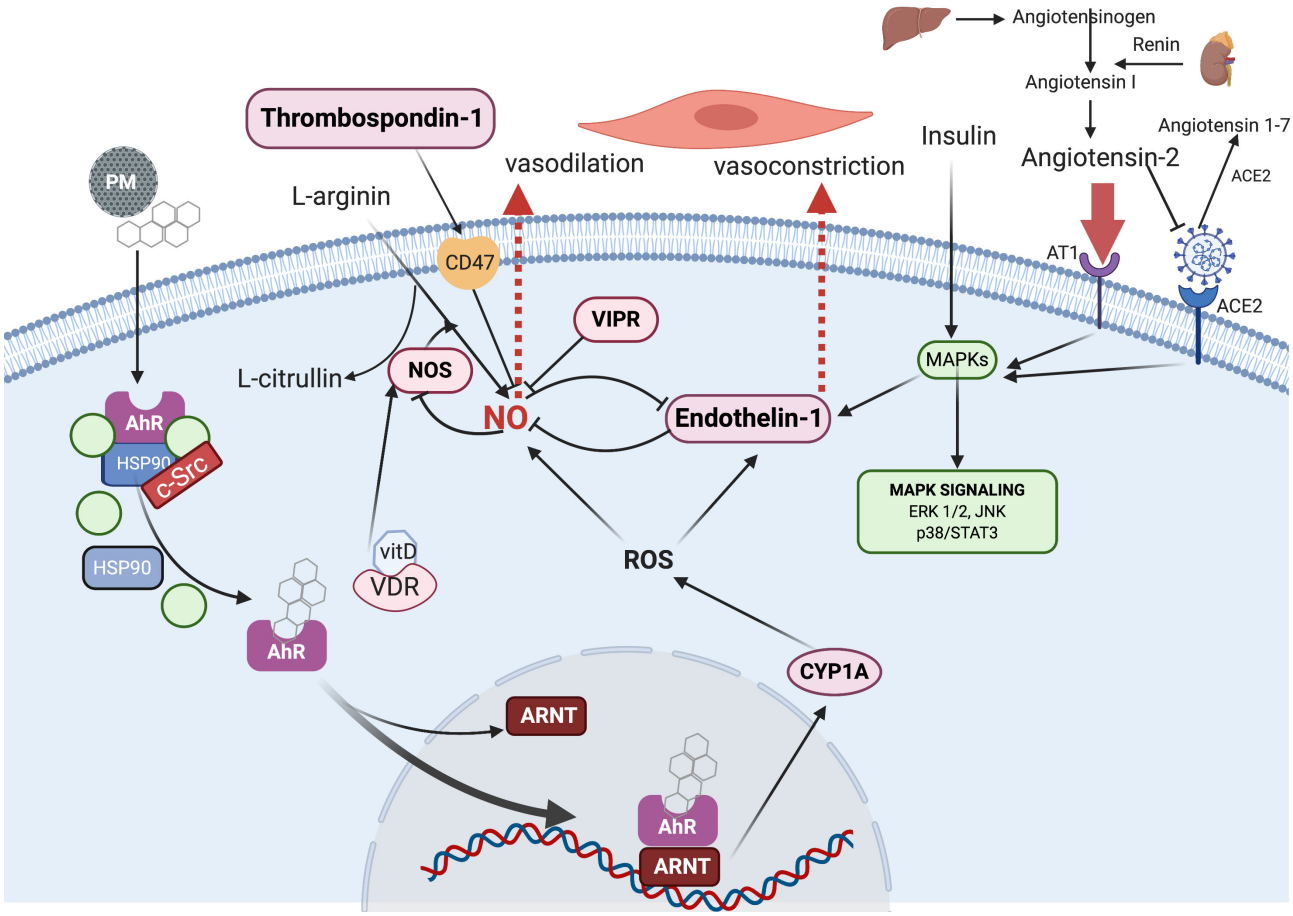
The increased blood circulation of endothelin-1 is considered a marker of risk for cardiovascular disease and has been correlated with ethnic differences in the severity of microvascular and macrovascular dysfunctions as well as with endothelial dysfunction related to aging.[116]

The endothelium homeostasis is maintained by a balance of vasoconstriction effects elicited by endothelin-1 and vasodilation operated by nitric oxide (NO). The control of this process is quite complex and has been reported to be of great importance in the development, progression and fate of COVID-19.

NO is a bioactive gas whose production in endothelium is coordinated by exogenous inputs, such as the dietary intake of L-arginine, and the endogenous action of various forms of nitric oxide synthase (NOS), among which iNOS is expressed as the consequence of inflammation, in response to cytokines. This process is regulated by the modulation of the thrombospondin-1 gene (TSP-1). TSP-1 is a potent angiostatic mediator.[118] It is able to activate transforming growth factor (TGF)-β, a potent pro-fibrotic and anti-inflammatory factor.[119] Its downregulation has been related to cardiovascular pathology, including myocardial infarction, heart failure, atherosclerosis, and aortic valve stenosis.[119] TSP-1 deficiency has been reported to worsen diabetic retinopathy, which is the leading cause of blindness worldwide.[118,120] The product of TSP-1 is a matricellular glycoprotein with a specific domain to bind membrane proteins such as integrin and the integrin-associated protein (CD47). The complex TSP-1 and CD47 regulates the cellular production of NO, eliciting an important function in vasodilation and chemotaxis.[121] At low doses NO is anti-inflammatory and anti-angiogenic. The increase of NO in response to inflammatory stimuli leads to leucocyte adhesion to endothelium, which is the first step to vascular dysfunction and disease.[3] The interaction of TSP-1 with two cell-surface receptors, CD36 and CD47, can inhibit the activation of NO, so blocking the adhesion and activation of leucocytes and preventing inflammation. To the contrary, as often in the case of other matricellular proteins, the freely circulating form of TSP-1, (i.e. when not receptor bound), has the opposite effect, increasing blood pressure.[121,122] Therefore, the TSP-1-mediated activation of NO is a delicate balance, where NO needs to be maintained at physiological levels, to ensure the appropriate regulation of the vascular tone and blood flow. It is clear that NO balance is also related to the integrity of RAAS axis. Indeed, endothelin-1 secretion is fostered by RAAS key molecule, angiotensin-II, by cytokines and by free radicals. NO can inhibit the production of endothelin-1. Conversely, endothelin-1 can inhibit NO production (figure 1).

The results from our study contribute further evidence that the disruption of this balance is likely due to the downregulation of all the key genes, TSP-1, iNOS, integrin, that are involved in the process (table 2). All these genes are negatively regulated by the concentration of NO in the cell. Therefore, we can speculate that either the exposure to PM increased the level of NO, triggering the NO negative feedback through the gene downregulation, or the upregulation of endothelin-1 inhibited iNOS, supporting the negative regulation of NO.

The combination of increased levels of endothelin-1 and low levels of NO has been proposed as a biomarker and a prognostic tool for individuals at risk to develop severe COVID-19, including ethnic groups, such as Afro-Americans or Hispanic-Latin, who appear to be at higher risk,[116] as do populations of South Asian origin,[123] and highlights the higher susceptibility to COVID-19 infection due to ethnic and/or genetic factors, that has been poorly explored



Pink icons represent genes that are dysregulated in T47D cells exposed to PM. / Le icone rosa rappresentano i geni che risultano disregolati nelle cellule T47D esposte a PM.

The increase of endothelin-1 levels, due to the upregulation of endothelin-1 gene, is the key event triggered by either PM exposure, through the formation of ROS via AhR canonical pathway, or SARS-CoV-2, through the modulation of MAPK signalling pathway. AhR can also activate MAPK signalling pathway, as reported in Mescoli 2020.[3] / L'aumento dei livelli di endotelina-1, dovuti alla sovraregolazione del gene endotelina-1, è l'evento chiave innescato dall'esposizione a PM, tramite formazione di ROS per la via canonica AhR, oppure a SARS-CoV-2, tramite la modulazione della via MAPK. AhR può essere attivato anche dalla via MAPK, come riportato in Mescoli 2020.[3]

Figure 1. Impact of PM exposure and SARS-CoV-2 infection on the regulation of nitric oxide production. (Created with BioRender.com)
Figura 1. Impatto dell'esposizione a PM e infezione da SARS-CoV-2 sulla regolazione della produzione di ossido nitrico. (Figura creata con BioRender.com)

so far. In addition to the high levels of endothelin-1 that characterize Afro-Americans and Hispanic-Latin populations, Asians are at higher risk of developing type 2 diabetes and show an increased prevalence of both diabetes and obesity mainly as a consequence of change from a more traditional diet and life style to a Western one.[123] Even if differences in socioeconomic status and living conditions, as well as the restricted access to healthcare services may be responsible for the higher rate of SARS-CoV-2 infection currently observed in these populations, their increased vulnerability may also be a combination of the higher rate of pre-existing comorbidities and specific genetic polymorphisms that can confer a higher susceptibility.

Additionally, VDR may be another important player in the complex regulation of NO. Literature reports suggest an interplay between the AhR and VDR. Indeed, CYP1A1 is involved in an alternative metabolic pathway of vitamin D and AhR itself has been described as a target of calcitriol, the active form of vitamin D.[124,125] The complex interaction between AhR and VDR is also involved in immune-mediated inflammation, leading to endothelium dysregulation, through the regulation of NO.[124] Through the regulation of NO, both AhR and VDR can affect the response to infections, as reported in animal studies.[126,127] NO had previously been reported to inhibit the replication of SARS-CoV in in vitro models.[128] Therefore, the use of exogenous NO has been proposed to alleviate the symptoms in COVID+ patients, suffering from acute respiratory distress syndrome and to contribute to better clinical outcomes.[129]

On the basis of our experimental in vitro model results, it is clear that several genes and gene pathways cooperate to maintain (or disrupt) the mechanism(s) of regulation of NO production.

One of the most interesting and intriguing results from our study is the modulation of VIP receptor1 gene, which is strongly upregulated after the exposure to PM (table 2). VIP is a potent neuropeptide controlling biological processes in almost all organs. VIP and its receptor play a role in metabolism, obesity, control of insulin release and several gastrointestinal disorders.[130] In lungs, VIP, via the activation of its receptors, controls the secretion of airway mucous.[131] The high expression of VIP receptors in smokers' lungs has been associated with chronic bronchitis.[131] Several cardiopulmonary disorders are associated with alterations in the levels of VIP or its receptors, due to a change in the VIP, in the VIP receptors or in the receptors affinity for their ligands. When VIP is produced at physiological con-

centrations and it binds to one of its coordinate receptors, it elicits anti-inflammatory, anti-fibrotic, inotropic, lusitropic, and vasodilatory effects.[132] Most effects are related to the VIP ability to regulate several pro-inflammatory cytokines, including TNF-α, IFN-γ, IL-12, IL-17A, and IL-6, which mediates the inflammatory response that are characteristic of the acute respiratory distress syndrome.[132] The vasodilatory effect of VIP has been also associated to increase in NO.[133] Indeed, high levels of VIP have been detected in plasma from patients who had survived severe COVID-19 and associated with VIP ability to decrease the production of pro-inflammatory cytokines.[134] To the contrary the up-regulation of VIP receptor may be related to the increase of pro-inflammatory cytokines levels.[132]

For their properties and efficacy in contrasting cardiopulmonary disorders, VIP receptors are the targets of two therapeutics, the synthetic VIP RLF-100 (Aviptadil) and the VIP receptor agonist PB1046 currently under evaluations in clinical trials as promising treatments in severe COVID-19.[135-137]

Finally, it needs to be noted again, that whilst our model is an appropriate mechanistic exploratory tool, experimental reproduction in lung in vitro models is needed to support and verify our molecular interpretation.

CONCLUSIONS

Based on the results obtained in our study, it is suggested that PM induces the modulation of genes that are involved in obesity, diabetes, and hypertension. Several of these genes, have been found to play a key role in severe COVID-19. Here we have specifically explored those related to glucose metabolism, diabetes, and obesity (sweet) with hypertension and cardiovascular disease (heart), such that we start to see the unwelcome hitchhiker has become a 'sweetheart' relationship. The analysis of the function of these particular genes in health and disease supports the evidence that obesity, hypertension, and diabetes are risk factors for developing severe COVID-19. Our results also suggest PM may play a role in strengthening the evidence for this risk, due to mediation by the same molecular targets. We postulate that the disruption of these particular targets is responsible for worsening pre-existing conditions, and for exacerbating the effects induced by SARS-CoV-2 infection.

Although the association between pre-existing conditions, environmental pollution, SARS-CoV-2 infectivity, and the course of COVID-19 in affected patients can be clarified only on the basis of appropriate epidemiological studies, the evidence that chemical agents and pathogens share key events at the molecular level provides new insights in the use of molecular signatures and pathway-based toxicity to predict adverse outcomes in human health.

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