SOCIETÀ ITALIANA DI FARMACOLOGIA

RELAZIONE DI METÀ PERIODO

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TIPOLOGIA DI BORSA RICEVUTA: Borsa di studio per l'Italia e per l'estero per progetti di ricerca in ambito farmacologico aventi per tema l'incentivazione delle ricerche sull'immuno-oncologia e sul ruolo del linfocita T nelle malattie autoimmunitarie (Borsa SIF BMS)

TIPOLOGIA DI RELAZIONE: Metà periodo

TITOLO DELLA RELAZIONE: Alteration of mitochondrial DNA: potential involvement in lung cancer establishment

RELAZIONE:

Background. Lung cancer is the leading cause of cancer related mortality worldwide (1). Every year, 1.8 million people have diagnosis of lung cancer, and 1.6 million die as a result of the disease. Although scientific advances in cancer knowledge have been made in the past decades, 5-year survival have low rate (4–17%) depending on stage and regional differences (1).
The environmental pollution (air pollution/particulate matter) exposure as well as lifestyle (primarily cigarette smoke) have been described as the major etiological risk factors associated with airway disease, particularly lung cancer (2). These factors are responsible of an increased genomic instability linked to mitochondrial oxidative DNA damage, as a result of an enhanced reactive oxygen species (ROS) production (3-4). In this context, 8-oxoguanine DNA glycosylase 1 (OGG1) is a DNA repair enzyme that excises 7,8-dihydro-8-oxoguanine (8oxoG), a mutagenic base which occurs as a result of exposure of DNA to ROS (5). Since the presence of 8oxoG is expression of highly mispairing lesion, probably due to decreased OGG1 expression level in mitochondria and cytosol, this condition could lead to higher frequency of mutation associated to lung cancer risk.
Based on these concepts, the overall goal of this project was to understand the link between the environmental pollution, cigarette smoke and potential alteration in terms of OGG1 expression, which deficiency is associated with an increased susceptibility to lung cancer (6).

Hypothesis. Our hypothesis was that the exposure to particulate matter was associated to an impaired OGG1 activity/expression in favor of mitochondrial oxidative DNA damage which may play a crucial role in tumorigenesis.

Experimental plan. The experimental protocol was performed in accordance with the guidelines and regulations provided and accepted by the Ethical Committee of the "Monaldi-AORN-Ospedale dei Colli" Hospital (approval number 1254/2014). For our purpose, we used peripheral blood mononuclear cells (PBMCs) from non-chronic obstructive pulmonary disease (COPD) and COPD patients. We used smokers

and COPD-derived PBMCs because it is well reported that smoking and COPD are a high-risk for lung cancer establishment, besides the fact that this pathology is induced by smoking (7-8). All COPD subjects were smokers or former smokers, non-COPD subjects were divided in smokers and non-smokers.
All subjects were 60±10 (mean±S.E.M.) years of age and had no history of allergic diseases. Blood was collected and used within 24 hours. PBMCs were isolated according to Ficoll's protocol (9), plated and treated with Nanoparticles of Organic Carbon (NOC, 50-100pg/ml) to mimic the exposition to environmental pollutants, at different experimental time points (1-3h).
8-hydroxy-2-deoxyguanosine (8-OHdG), a well-known marker for DNA damage derived from oxidative stress (10), was measured in the PBMCs cytosolic extract after 1h treatment with NOC. Mitochondrial OGG1 transcription and expression were measured by real-time polymerase chain reaction (RT-PCR) and western blotting, respectively, after 3h treatment with nanoparticles.

Results. In order to understand the effect of the environmental pollutants exposition and cigarette smoke on the human health, we measured the levels of 8-OHdG in the cytosolic extract of non-smokers-, smokers- and COPD-derived PBMCs after the treatment with ultrafine particles that mimic the environmental pollution. We observed that the addition of NOC significantly increased the release of 8-OHdG in both non-smokers and smoker-derived PBMCs. Similarly, we found that NOC induced high levels (four times higher than smokers) of 8-OHdG in COPD-derived PBMCs. According to these data all the three type of cohorts were subjected to DNA damage, although it was much more evident in COPD patients than healthy non-smokers and smokers. Therefore, we went on to analyze the levels of a repairing enzyme, OGG1, highly important to avoid DNA damage following oxidative stress (11). The administration of NOC significantly increased the levels of mRNA for OGG1 in PBMCs obtained from non-smokers, but this effect was not observed in smokers- and COPD-derived PBMCs. To note, we did not observe a significant increase in mtROS release from non-smokers after the addition of NOC compared to smokers and COPD patients, pointing out that smokers individuals could be more susceptible to mitochondria-based airway inflammation once exposed to air pollution.

Conclusion. These data show that ultrafine particles, such as NOC, which are sub-10nm particles in size, typical of environmental pollution, are able to induce mitochondria-derived oxidative stress, which is not countered by OGG1, enzyme involved in repairing mitochondria-derived oxidative stress, in smokers- and COPD-derived PBMCs compared to non-smokers individuals. Although, future studies are needed to understand cellular/molecular mechanism/s, these data provide new perspectives for the role of inhaled combustion particles that, together with other pulmonary insults, such as cigarette smoke, can lead to lung pathologies such as cancer.

References

1. Hirsch FR. et al. Lung cancer: current therapies and new targeted treatments. *Lancet* 389, 299–311 (2017).
2. Malhotra J et al. Risk factors for lung cancer worldwide. *Eur Respir J*. 48(3):889-902 (2016).
3. Fliss MS et al. Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. *Science*.287(5460):2017-9 (2000).
4. Grevendonk L et al. Mitochondrial oxidative DNA damage and exposure to particulate air pollution in mother-newborn pairs. *Environ Health*. 15:10. doi: 10.1186/s12940-016-0095-2 (2016).
5. Janssen K et al. DNA repair activity of 8-oxoguanine DNA glycosylase 1 (OGG1) in human lymphocytes is not dependent on genetic polymorphism Ser326/Cys326. *Mutat Res*. 486(3):207-16 (2001).
6. Xie Y et al. Deficiencies in mouse Myh and Ogg1 result in tumor predisposition and G to T mutations in codon 12 of the K-ras oncogene in lung tumors. *Cancer Res*. 64(9):3096-102 (2004).
7. Barreiro E et al. Relationships between chronic obstructive pulmonary disease and lung cancer: biological insights. *J Thorac Dis*. 8(10):E1122-E1135 (2016).
8. Colarusso C et al. Role of the inflammasome in chronic obstructive pulmonary disease (COPD). *Oncotarget*. (2017). doi: 10.18632/oncotarget.17850. [Epub ahead of print]

9. De Falco G et al. Human peripheral blood mononuclear cells (PBMCs) from smokers release higher levels of IL-1-like cytokines after exposure to combustion-generated ultrafine particles. *Sci Rep*. 7:43016 (2017).
10. Shimada K et al. Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity*. 36, 401–414 (2012).
11. Tumurkhuu G et al. Ogg1-Dependent DNA Repair Regulates NLRP3 Inflammasome and Prevents Atherosclerosis. *Circ. Res*. 119, e76-90 (2016).