Chapter

Monoamine Oxidase A (MAO-A): A Therapeutic Target in Lung Cancer

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Abstract

Monoamine oxidase-A (MAO-A), a pro-oxidative enzyme catalyzes the oxidative deamination of endogenous and exogenous monoamines/neurotransmitters like dopamine, serotonin, norepinephrine or tyramine and converting them into their corresponding aldehydes and reactive oxygen species (ROS). Hyperactivity of MAO-A has been shown to be involved in depression, neuro-degeneration including Parkinson's and Alzheimer's diseases, neuropsychiatric disorders and cardiovascular diseases. Our recent results however demonstrated the involvement of MAO-A in promoting aggressiveness of lung carcinoma. We found both constitutive and inducible expression of MAO-A in non-small cell lung cancer cells H1299 and in A549 lung epithelial carcinoma cells. By using knockout (by CRISPR-Cas9 gene editing technology) or knockdown (using MAO-A specific esiRNA) MAO-A cells we demonstrated the role of MAO-A in promoting lung cancer aggressiveness and epithelial to mesenchymal transition (EMT). From our observations, we can conclude that MAO-A may be considered as a potential therapeutic target for the intervention and treatment of lung carcinoma.

Keywords: monoamine oxidase-A (MAO-A), non-small cell lung carcinoma, 15-lipoxygenase, metastasis, epithelial to mesenchymal transition (EMT)

1. Introduction

Monoamine oxidase A (MAO-A) is a mitochondrial outer membrane-bound enzyme that catalyzes oxidative deamination of biogenic amines and subsequently generates reactive oxygen species (ROS) in the form of hydrogen peroxide (H_2O_2) as a catalytic by product. It is widely present in almost all mammalian cell types except in erythrocytes [1, 2].

It is well documented that abnormalities of MAO-A levels and activity can lead to neuropsychiatric disorders as it plays a vital role in the regulation of neurotransmitters. Moreover, MAO-A hyperactivity has been shown to be associated with depression and previous reports implicate MAO-A inhibitors as effective therapeutics against clinical depression and anxiety [3, 4]. Involvement of MAO-A has also been shown in neurodegenerative diseases including Parkinson's and Alzheimer's disease by inducing oxidative stress-mediated apoptosis [5, 6]. MAO-A deficiency and abnormal activity has also been associated with impulsive aggressive behavior [7], neuropsychiatric disorders [1], pancreatic beta cell function [8] and glucose

metabolism [9]. In addition to neurodegenerative disorders and neuroinflammatory diseases, mounting evidences have been suggested about the contribution of MAO-A in cardiovascular diseases like myocardial injury [10], heart failure [11], cardiac cell apoptosis [12] etc.

Previously it has been shown that MAO-A has a major contribution in the resolution of inflammation and thus been reported as a signature marker of alternatively activated monocytes/macrophages [13]. Reactive oxygen species (ROS) can predispose cancer cells to DNA damage and cause tumor initiation and progression [14]. This suggests that MAO-A might have a significant role in cancer. Rybaczyk et al. [15] carried out a study where they have analyzed a subset of cancer datasets concentrating on genes involved in the serotonergic pathway. Genechip datasets consisting of cancerous tissue from human, mouse, rat, or zebrafish were obtained from the GEO database [16, 17]. Initially, obvious changes that were common in various types of cancers were identified by comparing gene expression between cancerous tissues and normal tissues for each type of cancer. This study strongly demonstrated that MAO-A suppression could be linked to increased risk of cancer and MAO-A expression was decreased in 95.4% of human cancer patients and 94.2% of animal cancer cases compared to the non-cancerous controls [15].

In contrast, high Gleason grade or poorly differentiated prostate cancer exhibited increased MAO-A expression [18], and the increased level of MAO-A promoted prostate cancer metastasis [19, 20]. Furthermore, it was also reported that the overexpressed MAO-A in prostate cancer cells was the main causative agent for the reduced expression of E-cadherin and increased expression of vimentin and Twist at both mRNA and protein levels in prostate cancer [19]. These studies suggested a likely role of MAO-A for the progression of prostate cancer by mediating EMT. However, contradicting results were reported in case of HCC [21] and cholangiocarcinoma [22]. Therefore, it can be hypothesize from these reports that MAO-A functions across different cancer cells in a context specific manner and so, it is essential to further uncover the function of MAO-A in other cancers. All these reports indicate towards the emerging role of MAO-A in tumor growth, migration and metastasis. However, none of these studies revealed the role of MAO-A in lung cancer growth, migration and metastasis and its mechanistic regulation.

MAO-A can be present constitutively in many different types of cancer cells (like in H1299 lung cancer, HCT116 colorectal cancer or in LNCap prostate cancer cells), or it can be induced by Th2 cytokines IL-13/IL-4 in A549 lung epithelial carcinoma cell line or monocytic U937 cell line. In a very recent study by our group we showed that IL-13 mediated induction of MAO-A in human bronchial epithelial cell NHBE as well as in human lung carcinoma cell line A549. We also explored the mechanisms involved in the regulation of the expression/activity and function of MAO-A during IL-13-induction and presented evidence that Stat6, 15-LO and PPARγ are the critical regulators that are involved in regulating MAO-A gene expression and activity of A549 cells which further demonstrated the concerted mechanistic effects of these genes during IL-13-activation. Altogether, the IL-13/STAT6, STAT3, STAT1/15-LO/PPARγ signaling axis for regulating MAO-A gene expression and function add novel insights into the resolution of inflammation and in the progression of lung cancer [23]. In addition to that, our recent unpublished observation revealed that MAO-A plays an important role in regulating cancer cell aggressiveness and EMT transition. Moreover, a very recent report from Huang et al. has provided evidence that MAO-A plays a key role in EMT and HIF-1α protein accumulation induced by HPV-16 E7 in NSCLC cells, suggesting that MAO-A may be a potential therapeutic target for HPV-related NSCLC [24]. So, based on these recent findings, we have focused on the potential contribution of MAO-A in lung cancer aggressiveness and metastasis and EMT transition. Previous literature and

our observations thus indicated that MAO-A could serve as a potential therapeutic target of lung cancer intervention and treatment.

In this chapter, we precisely highlighted the contribution of MAO-A in lung cancer aggressiveness and EMT and thus finally to the prognosis of the lung cancer patients primarily based on the observations obtained from our recent publication and ongoing research work and background studies. We also tried to explore the fact that MAO-A being a well-known contributor in neurological and neuropsychiatric disorders, how it could also be a tempting target of lung cancer treatment.

2. MAO-A is co-induced with 15-LO in human lung cancer cells during IL-13 activation

15-lipoxygenase (15-LO) is a lipid peroxidating enzyme which is substantially induced in human peripheral blood monocytes after IL-4/IL-13 activation. This enzyme oxidizes polyunsaturated fatty acids like linoleic and arachidonic acids to their corresponding hydroperoxides like 13-S-HPODE and 15-S-HPETE [23, 24], which have been implicated as inflammatory mediators in cell development and in the pathogenesis of various diseases [25–29].

In a very recent study, our group demonstrated that MAO-A is co-induced with 15-LO in monocytes/macrophages, normal human bronchial epithelial (NHBE) cells and in A549 lung epithelial carcinoma cell line in response to IL-13 treatment [23]. Moreover, concordant 15-LO and MAO-A induction following IL-13 stimulation was also investigated in other lung epithelial cancer cells like in H1299 NSCLC. In H1299 cells, 15- LO expression level was very low and was not induced by IL-13. In contrast, MAO-A was constitutively present in H1299 cells but was not further induced upon IL-13 stimulation. These results thus demonstrate that in H1299 NSCLC, MAO-A is already overexpressed and no further IL-13-dependent induction occurs in these cells.

3. Transcriptional regulation of MAO-A and 15-LO upon IL-13 induction

In previous reports, it was demonstrated that Stats (Stat1, Stat3 and Stat6) are required for controlling IL-13- mediated 15-LO and MAO-A gene expression [30, 31]. Along with the same line, our recent research article further confirmed the direct binding of Stat transcription factors (Stat1, Stat3 and Stat6) to their cognate DNA binding sites present in the 15- LO promoter after IL-13 stimulation in primary monocytes [23]. On the other hand, predicted transcription factor binding sites of MAO-A promoter does not show any Stat consensus sequence in its promoter but reveals presence of a bunch of different other transcription factor binding sites like Sp1, GATA, TBP and GRE [32]. To justify and validate the prediction of different transcription factor binding sites located in the MAO-A promoter, we pursued experiment and presented evidence that in response to IL-13 stimulation, Sp1 transcription factor directly binds to the cognate DNA binding sites in the MAO-A promoter after IL-13 stimulation in primary monocytes [23]. After establishing the role of Sp1 trascription factor in regulation of MAO-A activity, now we are trying to explore the role of other transcription factors like GATA, TBP, GRE in the regulation of MAO-A.

It was previously demonstrated by our group that as Egr-1 and CREB binding sites are present in 15-LO promoter, in case of primary human monocytes, Egr-1 and CREB explicitly bind to their cognate sequences upon IL- 13 induction [33].

In addition to that, our data also affirmed that there are two distinct diverged imitate signaling pathways downstream of the IL-13 receptor that regulate 15-LO gene expression in primary monocytes [33]. Our study revealed that other than the conventional IL-4Rα-Jak2-Stat3-dependent pathway [34], there exists a IL-13Rα1-Tyk2-mediated pathway which is vital for IL-13-induced Egr-1 and CREB activation via MEK-ERK1/2. So, transcription factors Egr-1 and CREB plays a very crucial role in IL-13-induced 15-LO gene expression in primary human monocytes [33]. Based on these previous reports, and as 15-LO and MAO-A genes are co-induced upon exposure to IL-13 during alternative activation of monocytes, we further asked a question that whether Egr-1 and CREB transcription factors have any role in regulating IL-13- stimulated MAO-A gene expression in primary monocytes. Our experimental data strongly supported the regulatory role of transcription factors Egr-1 and CREB in mediating IL-13-stimulated MAO-A gene expression in primary monocytes probably by inducing 15-LO expression.

Therefore, collectively, the results presented previously by our group and in our recent study strongly suggest that Stats (Stat1, Stat3 and Stat6) as well as Egr-1 and CREB transcription factors are critical regulators of MAO-A activity in alternatively activated monocytes by IL-13.

Considering the fact that both 15-LO and MAO-A are co-induced upon IL-13 stimulation in primary human monocytes/macrophages and in A549 cells, and since activity of both of them are regulated by the transcriptional activation of several Stats (Stat1, Stat3 and Stat6), Egr-1 and CREB in monocyte/macrophage and in A549 cells, we further intended to explore whether one of them is the upstream regulator of another. As expected, experimental results the fact that IL-13- induced gene expression in lung carcinoma cell line A549 15-LO dependent [23].

4. IL-13-induced MAO-A contributes in lung cancer cell aggressiveness

It was previously reported that increased expression of MAO-A is associated with high grade aggressive prostate cancer [18, 35]. The ability of MAO-A to induce EMT in prostate cancer cells results in increased migratory, proliferative, invasive and metastatic potential through an elevation of ROS [19]. MAO-A-generated ROS modulates HIF1α (a master regulator of hypoxia) activity by suppressing PHD (oxygen dependent prolyl hydroxylases) activity. It was further verified that MAO-A enzymatic activity rather than the protein expression which is responsible for enhanced level of migration, invasion and proliferation of prostate cancer cells by the induction of EMT. These results thus suggest that MAO-A expression in high-grade tumors might have a likely role in maintaining a dedifferentiated phenotype and promoting aggressive behavior. Based on these previous reports, our group also investigated the contribution of MAO-A in enhancing the aggressiveness of different type of cancer cells like H1299 lung cancer cells and in HCT116 colorectal cancer cells where MAO-A is constitutively expressed and is responsible for promoting migration and invasion of these cancer cells (unpublished observations by our group). Based on our current observations, we also hypothesized that inducible MAO-A expression in A549 cells by IL-13/IL-4 might be involved in ling cancer progression and metastasis.

15-LO products HPODE/HPETE are well known PPARγ ligands and in our recent study, we have confirmed that IL-13 expression of MAO-A in lung epithelial carcinoma cell line A549 is dependent upon PPARγ [23]. Moreover, in our recent study, we have further confirmed that IL-13 stimulated A549 cell migration is mediated by MAO-A and requires both 15-LO and PPARγ activity. *In vitro* transwell

migration assay using the MAO-A activity inhibitor Moclobemide along with the 15-LO inhibitor PD146176 and PPARγ antagonist GW9662 confirmed the above observation. In presence of Moclobemide, IL-13-induced A549 cell migration was reduced substantially whereas Moclobemide alone in absence of IL-13 showed no change compared to the unstimulated control, thereby suggesting a plausible role of MAO-A in A549 tumor cell migration *in vitro*. Similarly, significant reduction of migration was also observed in IL-13-activated A549 cells after treatment with

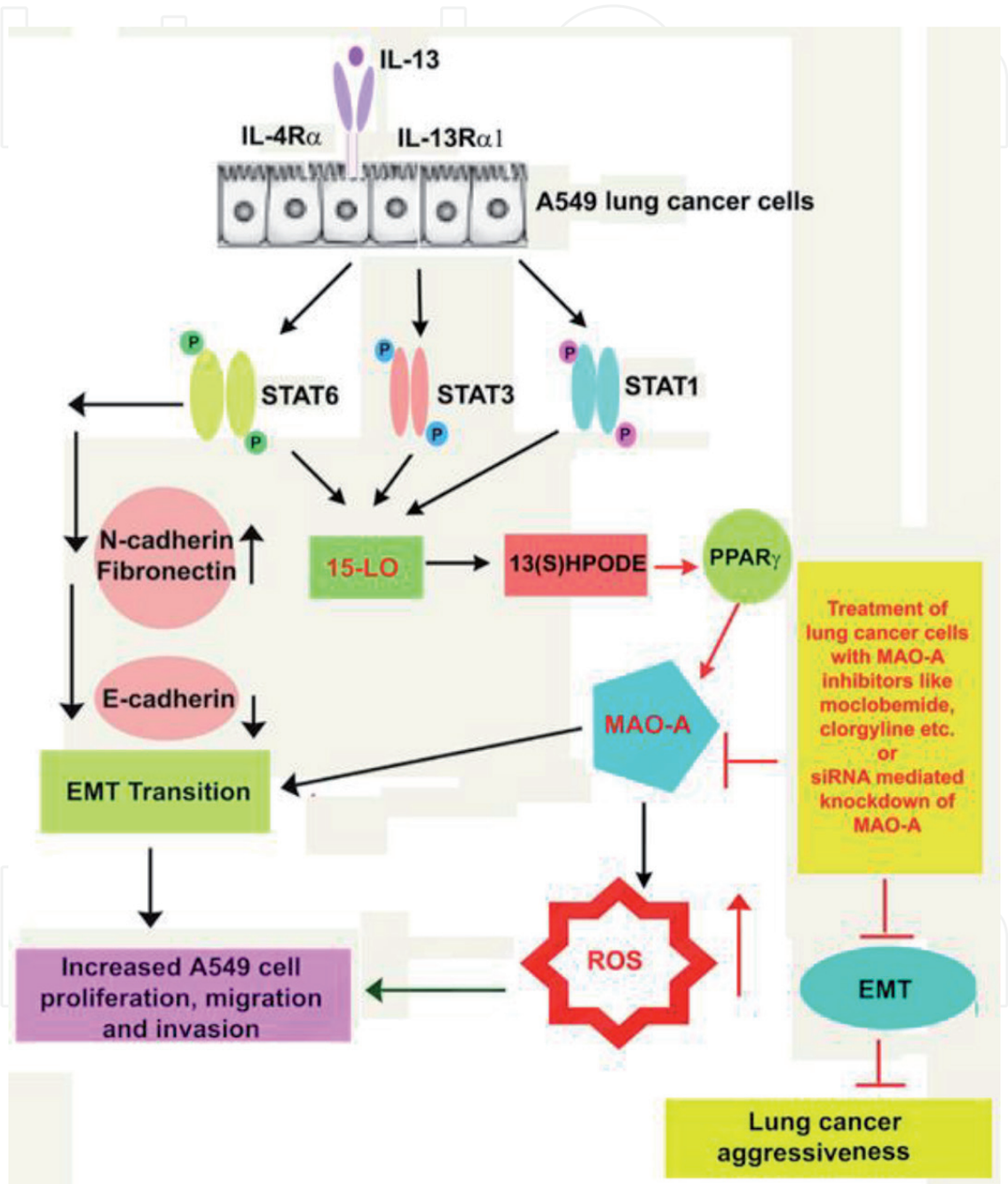

Figure 1.
Proposed mechanism of how IL-13 induced MAO-A cancer cell aggressiveness in lung cancer. IL-13-mediated activation of MAO-A expression/activity in A549 lung epithelial carcinoma cells is activated by IL-13/ (STAT6, STAT3, STAT1)/15-LO/PPARγ signaling axis and expression/activity of this induced MAO-A mediates ROS generation which is believed to have significant role on A549 cell migration, invasion and proliferation which are all associated with the aggressiveness of this particular cancer cell. Moreover, treatment of A549 cells with MAO-A specific inhibitors like moclobemide or clorgyline or esiRNA- mediated knockdown of MAO-A gene in A549 cells showed significant downregulation in the migration, invasion and proliferation of lung cancer cells. This finding marked MAO-A as a promising therapeutic target for aggressive lung cancer treatment. IL-13: Interleukin 13, MAO-A: Monoamine oxidase A, STAT6: Signal transducer and activator of transcription 6, 15-LO: 15 lipoxygenase, PPARγ: Peroxisome proliferator-activated receptor gamma.

the 15-LO activity inhibitor PD146176 and PPARγ antagonist GW9662 in a dose-dependent manner compared to IL-13-stimulated positive control [23]. Moreover, we further confirmed the role of MAO-A in lung cancer cell migration and invasion in either MAO-A knockdown cells (using MAO-A esiRNA specific gene silencing) or MAO-A knockout cells (by using CRISPR-Cas9 gene editing technology) [Unpublished observations by our group].

Recently it was reported that the Th2 cytokine IL-13 contributes crucially in promoting EMT and enhancing aggressiveness (migration and invasion) in colorectal cancer (CRC) cells (HT29 and SW480 cells by triggering IL-13/IL-13Rα1/STAT6/ZEB1 signaling axis) [36]. Recent findings from our group further suggest that IL-13/IL-13Rα1/Stat6 signaling axis is involved in regulating the expression/activity of MAO-A in A549 lung epithelial carcinoma cells via a 15-lipoxygenase (15-LO)-dependent process involving PPARγ [23] which may be the main reason of promoting migratory, invasive and metastatic potential of the cancer cells (unpublished observations). But these results further need to be validated in *in-vivo* model or by using high grade lung cancer patient tissue samples to conclusively comment on the role of MAO-A on cancer progression and metastasis. The mechanism by which MAO-A lung cancer cell aggressiveness is described under a schematic representation in **Figure 1**.

5. MAO-A plays an important role in epithelial to mesenchymal transition (EMT) in lung carcinoma

EMT is a vital and commutative process, during which epithelial cells transit from polarized, cobble stone like cells to migratory, spindle-shaped mesenchymal cells. Apart from the morphological changes, changes at the molecular level by losing expression of various epithelial markers such as E-cadherin, ZO-1 and occludin, and acquiring expression of mesenchymal markers including N-cadherin, vimentin, and fibronectin are also very common in the cells experiencing EMT [37, 38].

Various studies have suggested a robust correlation between different EMT markers like E-cadherin, hypoxia inducible factor 1α (HIF-1α), twist, snail and poor prognosis in lung cancer [39]. Specially, in case of NSCLC, expression of Twist, Slug, and Foxc2 was identified as important marker of recurrence-free and overall survival in stage I NSCLC [40]. High expression pattern of various EMT related markers have been identified in advanced primary lung cancer specimens, particularly in squamous cell carcinoma [41]. Reduced EMT markers expression were observed in case of brain metastasis to primary NSCLC, supporting the notion that disseminated tumor cells undergo EMT at the site of metastasis [42, 43]. It was also suggested that enhanced expression of Forkhead box M1 (FOXM1), a member of the Fox family of factors, may have prognostic value for patients with NSCLC, and FOXM1 was shown to promote metastasis by inducing EMT through activation of the AKT/p70S6K signaling axis [44].

Plethora of earlier reports suggested that MAO-A-mediated generation of excessive intracellular level of hydrogen peroxide, a major ROS species can induce epithelial to mesenchymal transition (EMT) in cancer cells. An extensive study by Wu et al. in 2014 [19] elaborately described how MAO-A affects prostate cancer cell (PCa) growth and metastasis and demonstrated for the first time, that MAO-A induces EMT and augments hypoxic responses to increase the migratory, invasive, and metastatic potential of PCa cells.

In a recent study, Liu et al. [45] determined the expression of MAO-A and different EMT markers in 45 pairs of NSCLC and matched non-tumor adjacent lung tissues to further explore the connection between MAO-A expression and the EMT

or the development of clinicopathological characteristics. From the results it was observed that both the protein and mRNA expression levels of MAO-A in NSCLC tissues were higher than those observed in the matched non-tumor adjacent lung tissues. Furthermore, in correlation with the previous notion, the enhanced expression of MAO-A in NSCLC tissues was positively associated with N-cadherin, Slug, and Twist, but negatively with E-cadherin expression. Furthermore, the elevated MAO-A expression in NSCLC tissues was also related with late stage NSCLC ($Z = -2.596$, $P = 0.029$) and lymph node metastases ($Z = -2.378$, $P = 0.020$). These findings indicated that MAO-A may have a role in inducing NSCLC progression by mediating EMT.

Next, considering the fact that high expression of monoamine oxidase A (MAO-A) in non-small cell lung cancer (NSCLC) is related to epithelial-mesenchymal transition (EMT) and the development of clinicopathological features of NSCLC, very recently, Yang et al. [46] tried to evaluate the role of a previously synthesized MAO-A inhibitor (G11) on inhibiting paclitaxel resistant NSCLC metastasis and growth. Experimental results showed that G11 significantly abrogated the viability of paclitaxel (PTX)-resistant NSCLC cell lines (A549/PTX and H460/PTX). G11 also abrogated the expression of MAO-A in A549/PTX and H460/PTX cells, which displayed relatively high MAO-A expression levels. Moreover, G11 was found to impede A549/PTX and H460/PTX cell migration and invasion. Furthermore, the *in-vivo* study also suggested that the co-administration of G11 and paclitaxel significantly suppressed tumor metastasis in H460/PTX lung metastasis models.

Considering these reports, we checked the expression of different EMT related markers like E-cadherin, N-cadherin, twist, snail, vimentin, etc. in MAO-A esiRNA treated A-549 cells as well as MAO-A knockout A549 cell line (by using CRISPR-Cas9 gene editing technology). As expected, these data confirmed regulatory role of MAO-A in EMT in lung carcinoma [Our unpublished observations]. Now to further validate the role of MAO-A in EMT and cancer metastasis, we are trying to explore the status of MAO-A, 15-LO and different EMT markers in lung cancer patient samples.

So, collectively, mounting evidences from different research reports and our recent observation strongly recommend further investigations for MAO-A as a tempting therapeutic target for lung cancer treatment.

6. Discussion

Monoamine oxidase A, an enzyme responsible for the oxidative deamination of biogenic amines, is well-known to be closely associated with impulsive aggressive behavior, anxiety, depression, and is considered as an indicator of psychological status [47, 48]. Recently, several studies have been focusing on the relationship between MAO-A expression and cancers [18–20, 49]. Initially, increased MAO-A expression was reported in high-grade aggressive prostate cancer, it was also demonstrated that increased expression of MAO-A was capable of mediating prostate tumorigenesis and metastasis [18–20]. Recently, MAO-A was reported as a novel decision maker in apoptosis and autophagy processes occurring within hormone refractory neuroendocrine prostate cancer cells [29]. Moreover, clorgyline, a known MAO-A inhibitor, was found to display anti-oncogenic and pro-differentiation effects on high-grade prostate cancer cells [50]. In contrary, MAO-A inhibitor-near-infrared dye conjugate was reported to reduce prostate tumor growth [51]. These findings suggest a likely role of MAO-A in mediating prostate cancer progression. However, in contrast to the previous reports of prostate cancer cells, Li et al. demonstrated that MAO-A expression was appreciably downregulated in clinical

HCC tissue samples [18], and MAO-A subdued HCC metastasis by hindering the adrenergic system and its transactivation of EGFR signaling [21]. Huang et al. also found that MAO-A expression was impeded by coordinated epigenetic and IL-6-driven events in human cholangiocarcinoma [22], and that overexpression of MAO-A suppressed cholangiocarcinoma growth and invasion [22].

So, from the above discussion it is clear that MAO-A level is regulated in different cancer cell lines in context specific manner. Moreover, MAO-A expression in high grade tumors may play a crucial role in promoting aggressive behavior of cancer cells. MAO-A degrades monoamine neurotransmitters by oxidative deamination and produces ROS. Increased level of ROS generation can be an important inducer of tumorigenesis, progression and metastasis in high grade cancers. Hence enhanced level of MAO-A expression and aggressive behavior of cancer cells may be correlated in advanced grade of cancer. From the studies on different type of cancer, it is quite evident that MAO-A may serve as a diagnostic biomarker and can also be applied as a therapeutic target in the treatment of cancer.

Furthermore, in case of NSCLC, as we have discussed earlier, research article by Liu et al. [45] supported our findings that MAO-A expression was significantly increased in NSCLC tissues, which was positively associated with EMT, late stages and lymph node metastases of the cancer, thus supporting the notion that MAO-A may play a role in NSCLC progression by regulating the EMT process.

In addition to that, along with the same line, Yang et al. [46] very recently established the role of MAO-A in lung cancer cell metastasis and EMT transition. So, these findings strongly recommend MAO-A as a promising therapeutic target of lung cancer treatment.

In our recent report, our result has demonstrated that IL-13- induced A549 cell migration was significantly downregulated in presence of moclobemide, a indicating a role of moclobemide in regulating lung cancer cell aggressiveness [23]. Wang et al. [52] that targeting MAO-A with FDA approved antidepressants could be a promising treatment option for the prostate cancer. It was reported by them that the antiandrogen enzalutamide (Enz) has improved survival in castration resistant prostate cancer (CRPC) patients. However, most patients eventually develop Enz resistance inducing by the androgen receptor (AR) splicing variant 7 (ARv7). Experimental results demonstrated that elevated expression of monoamine oxidase-A (MAO-A) is correlated with positive ARv7 detection in CRPC patients upon Enz treatment. Targeting MAO-A with phenelzine or clorgyline, the FDA-approved drugs for antidepression, resensitize the Enz resistant (EnzR) cells to Enz treatment and further subdudes EnzR cell growth *in-vitro* and *in-vivo*.

Moreover, Lee et al. [53] in 2013 have demonstrated that in case of LnCaP-LN3 prostate cancer cells MAO-A inhibitor pargyline significantly induced cell cycle arrest at the G1 phase compared to the control cells. In addition, pargyline induced an increase in the cell death rate by promoting apoptosis. Clinical depression is a very common feature in prostate cancer and mounting evidences have suggested that MAO-A levels are frequently elevated in different cancer types and MAO-A inhibitors (which are basically antidepressants) can serve as repurposing drugs for the treatment of cancer. Zarmouh et al. have identified a novel flavonoid MAO-A inhibitor which shows antiproliferative effect on prostate cancer cells [54].

Our recent research and different other research articles, it is well established that selective inhibitors of MAO-A like moclobemide, clorgyline, pargyline or a novel synthetic flavonoid can efficiently reduce cancer cell aggressiveness by either inhibiting cancer cell migration, proliferation or promoting apoptosis. So, selective inhibitors of MAO-A could also be used as a promising therapeutic agents for lung cancer treatment.

Moreover, in our unpublished observation, esiRNA specific knockdown of MAO-A in lung cancer cell A549 have shown significant downregulation of cancer cell

migration, invasion and EMT transition. So, siRNA-mediated gene silencing approach for MAO-A could also be used as a potential therapeutic approach in lung cancer treatment.

7. Conclusion

In cancer cells, migration and invasion are the basic steps that control metastasis which is a principle cause of cancer-related death. Recent reports demonstrate that MAO-A is involved in promoting prostate cancer progression by inducing epithelial to mesenchymal transition (EMT) which ultimately causes set up of ROS, thus increasing the ability of migration and invasion of these cells. MAO-A enzymatic activity is shown to be the main causative agent for MAO-A-driven ROS generation in cancer cells which acts as the critical regulator of MAO-A-mediated functions like migration, invasion and proliferation.

Evidences from different reports and our own findings suggest that elevated levels of MAO-A is a key feature of advanced stage of lung carcinoma and plays a crucial role in lung cancer cell aggressiveness through induction of epithelial to mesenchimal transition. Thus it is really novel to report that an oxidative enzyme which is preliminarily found to be involved in depression and antisocial behavior now can be considered as a biomarker for lung cancer therapy.

Altogether, our results strongly support that MAO-A can be used as a potential therapeutic target of lung cancer treatment for the better prognosis of the disease.

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References

[1] Shih JC, Chen K, Ridd MJ. Monoamine oxidase: From genes to behavior. Annual Review of Neuroscience. 1999;**22**:197-217

[2] Shih JC, Wu JB, Chen K. Transcriptional regulation and multiple functions of MAO genes. Journal of Neural Transmission. 2011;**118**(7):979-986

[3] Meyer JH, Ginovart N, Boovariwala A, Sagrati S, Hussey D, Garcia A, et al. Elevated monoamine oxidase a levels in the brain: An explanation for the monoamine imbalance of major depression. Archives of General Psychiatry. 2006;**63**:1209-1216

[4] Schwartz TL. A neuroscientific update on monoamine oxidase and its inhibitors. CNS Spectr 2013; Suppl 1: 25-32.

[5] Naoi M, Maruyama W, Akao Y, Yi H, Yamaoka Y. Involvement of type a monoamine oxidase in neurodegeneration: Regulation of mitochondrial signaling leading to cell death or neuroprotection. Journal of Neural Transmission. Supplementum. 2006;**71**:67-77

[6] Ou XM, Chen K, Shih JC. Monoamine oxidase a and repressor R1 are involved in apoptotic signaling pathway. Proc Natl Acad Sci USA. 2006;**103**:10923-10928

[7] Brunner HG, Nelen M, Breakefield XO, Ropers HH, van Oost BA. Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase a. Science. 1993;**262**:578-580

[8] Adeghate E, Parvez H. The effect of diabetes mellitus on the morphology and physiology of monoamine oxidase in the pancreas. Neuro Toxicol. 2004;**25**:167-173

[9] Panagiotidis G, Lindstrom P, Stenstrom A, Lundquist I. Glucose modulation of islet monoamine oxidase activity in lean and obese hyperglycemic mice. Metabolism. 1993;**42**:1398-1404

[10] Bianchi P, Kunduzova O, Masini E, Cambon C, Bani D, et al. Oxidative stress by monoamine oxidase mediates receptor-independent cardiomyocyte apoptosis by serotonin and postischemic myocardial injury. Circulation. 2005;**112**:3297-3305

[11] Kaludercic N, Carpi A, Menabo R, Di Lisa F, Paolocci N. Monoamine oxidases (MAO) in the pathogenesis of heart failure and ischemia/reperfusion injury. Biochimica et Biophysica Acta. 1813;**2011**:1323-1332

[12] Pchejetski D, Kunduzova O, Dayon A, Calise D, Seguelas MH, et al. Oxidative stress-dependent sphingosine kinase-1 inhibition mediates monoamine oxidase A-associated cardiac cell apoptosis. Circulation Research. 2007;**100**:41-49

[13] Cathcart MK, Bhattacharjee A. Monoamine oxidase a (MAO-A): A signature marker of alternatively activated monocytes/macrophages. Inflamm Cell Signal. 2014;**1**:152-159

[14] Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: A radical therapeutic approach? Nature Reviews. Drug Discovery. 2009;**8**:579-591

[15] Rybaczyk LA, Bashaw MJ, Pathak DR, Huang K. An indicator of cancer: Downregulation of monoamine oxidase-a in multiple organs and species. BMC Genomics. 2008;**9**:134

[16] Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, et al. Large-scale meta-analysis of cancer microarray data identifies common

transcriptional profiles of neoplastic transformation and progression. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:9309-9314

[17] NCBI Gene Expression Omnibus profiles http://www.ncbi.nlm.nih.gov/sites/entrez?db=geo

[18] Peehl DM, Coram M, Khine H, Reese S, Nolley R, et al. The significance of monoamine oxidase-a expression in high grade prostate cancer. The Journal of Urology. 2008;**180**:2206-2211

[19] Wu JB, Shao C, Li X, Li Q, Hu P, et al. Monoamine oxidase a mediates prostate tumorigenesis and cancer metastasis. The Journal of Clinical Investigation. 2014;**124**:2891-2908

[20] Wu JB, Yin L, Shi C, Li Q, Duan P, et al. MAOA-dependent activation of Shh-IL6-RANKL signaling network promotes prostate cancer metastasis by engaging tumor-stromal cell interactions. Cancer Cell. 2017;**31**:368-382

[21] Li J, Yang XM, Wang YH, Feng MX, Liu XJ, et al. Monoamine oxidase a suppresses hepatocellular carcinoma metastasis by inhibiting the adrenergic system and its transactivation of EGFR signaling. Journal of Hepatology. 2014;**60**:1225-1234

[22] Huang L, Frampton G, Rao A, Zhang KS, Chen W, et al. Monoamine oxidase a expression is suppressed in human cholangiocarcinoma via coordinated epigenetic and IL-6-driven events. Laboratory Investigation. 2012;**92**:1451-1460

[23] Dhabal S, Das P, Biswas P, Kumari P, Yakubenko VP, et al. Regulation of monoamine oxidase a (MAO-A) expression, activity and function in IL-13-stimulated monocytes and A549 lung carcinoma cells. The Journal of Biological Chemistry. 2018;**293**:14040-14064

[24] Huang B, Zhou Z, Liu J, Wu X, Li X, et al. The role of monoamine oxidase a in HPV-16 E7-induced epithelial-mesenchymal transition and HIF-1α protein accumulation in non- small cell lung cancer cells. International Journal of Biological Sciences. 2020;**16**:2692-2703

[25] Yamamoto S. Mammalian lipoxygenases: Molecular structures and functions. Biochimica et Biophysica Acta. 1992;**1128**:117-131

[26] Wittwer J, Hersberger M. The two faces of the 15-lipoxygenase in atherosclerosis. Prostaglandins, Leukotrienes, and Essential Fatty Acids. 2007;**77**:67-77

[27] Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation:Dual anti-inflammatory and pro-resolution lipid mediators. Nature Reviews. Immunology. 2008;**8**:349-361

[28] Cathcart MK, Folcik VA. Lipoxygenases and atherosclerosis:Protection versus pathogenesis. Free Radical Biology & Medicine. 2000;**28**:1726-1734

[29] Ku¨hn H, O'Donnell VB. Inflammation and immune regulation by 12/15-lipoxygenases. Progress in Lipid Research 2006; 45: 334-356.

[30] Bhattacharjee A, Shukla M, Yakubenko VP, Mulya A, Kundu S, Cathcart MK. IL- 4 and IL-13 employ discrete signaling pathways for target gene expression in alternatively activated monocytes/macrophages. Free Radical Biology & Medicine. 2013;**54**:1-16

[31] Xu B, Bhattacharjee A, Roy B, Xu HM, Anthony D, et al. Interleukin-13 induction of 15-lipoxygenase gene expression requires p38

mitogen-activated protein kinase mediated serine 727 phosphorylation of Stat1 and Stat3. Molecular and Cellular Biology. 2003;**23**:3918-3928

[32] Gupta V, Khan AA, Sasi BK, Mahapatra NR. Molecular mechanism of monoamine oxidase a gene regulation under inflammation and ischemia-like conditions: Key roles of the transcription factors GATA2, Sp1 and TBP. Journal of Neurochemistry. 2015;**134**:21-38

[33] Bhattacharjee A, Mulya A, Pal S, Roy B, Feldman GM, Cathcart MK. Monocyte 15-lipoxygenase gene expression requires ERK1/2 MAPK activity. Journal of Immunology. 2010;**185**:5211-5224

[34] Bhattacharjee A, Xu B, Frank DA, Feldman GM, Cathcart MK. Monocyte 15- lipoxygenase expression is regulated by a novel cytosolic signaling complex with protein kinase C delta and tyrosine-phosphorylated Stat3. Journal of Immunology. 2006;**177**:3771-3781

[35] True L, Coleman I, Hawley S, Huang CY, Gifford D, et al. A molecular correlate to the Gleason grading system for prostate adenocarcinoma. Proc Natl Acad Sci USA. 2006;**103**:10991-10996

[36] Cao H, Zhang J, Liu H, Wan L, Zhang H, et al. IL-13/STAT6 signaling plays a critical role in the epithelial-mesenchymal transition of colorectal cancer cells. Oncotarget. 2016;**7**:61183-61198

[37] Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nature Reviews. Molecular Cell Biology. 2014;**15**:178-196

[38] Said NA, Williams ED. Growth factors in induction of epithelial-mesenchymal transition and metastasis. Cells, Tissues, Organs. 2011;**193**:85-97

[39] Xiao D, He J. Epithelial mesenchymal transition and lung cancer. Journal of Thoracic Disease. 2010;**2**:154-159

[40] Jiang W, Pang XG, Wang Q, Shen YX, Chen XK, Xi JJ. Prognostic role of twist, slug, and Foxc2 expression in stage I non-small-cell lung cancer after curative resection. Clinical Lung Cancer. 2012;**13**:280-287

[41] Prudkin L, Liu DD, Ozburn NC, Sun M, Behrens C, et al. Epithelial-to-mesenchymal transition in the development and progression of adenocarcinoma and squamous cell carcinoma of the lung. Modern Pathology. 2009;**22**:668-678

[42] Lim J, Thiery JP. Epithelial-mesenchymal transitions: Insights from development. Development. 2012;**139**:3471-3486

[43] Gao D, Joshi N, Choi H, Ryu S, Hahn M, et al. Myeloid progenitor cells in the premetastatic lung promote metastases by inducing mesenchymal to epithelial transition. Cancer Research. 2012;**72**:1384-1394

[44] Kong FF, Qu ZQ, Yuan HH, Wang JY, Zhao M, et al. Overexpression of FOXM1 is associated with EMT and is a predictor of poor prognosis in non-small cell lung cancer. Oncology Reports. 2014;**31**:2660-2668

[45] Liu F, Hu L, Ma Y, Huang B, Xiu Z, et al. Increased expression of monoamine oxidase a isassociated with epithelial to mesenchymal transition andclinicopathological features in non-small cell lung cancer. Oncology Letters. 2018;**15**:3245-3251

[46] Yang X, Zhao D, Li Y, Li Y, Cui W, et al. Potential monoamine oxidase a inhibitor suppressing paclitaxel-resistant non-small cell lung cancer metastasis and growth. Thoracic Cancer. 2020. DOI: 10.1111/1759-7714.13617

[47] Liu Z, Huang L, Luo XJ, Wu L, Li M. MAOA variants and genetic susceptibility to major psychiatric disorders. Molecular Neurobiology. 2016;**53**:4319-4327

[48] Voltas N, Aparicio E, Arija V, Canals J. Association study of monoamine oxidase-a gene promoter polymorphism (MAOA-uVNTR) with self-reported anxiety and other psychopathological symptoms in a community sample of early adolescents. Journal of Anxiety Disorders. 2015;**31**:65-72

[49] Biswas P, Dhabal S, Das P, Das P, Swaroop S, et al. Role of monoamine oxidase a (MAO-A) in cancer progression and metastasis. Cancer Cell & Microenvironment. 2018;**5**:e1623

[50] Zhao H, Flamand V, Peehl DM. Anti-oncogenic and pro-differentiation effects of clorgyline, a monoamine oxidase a inhibitor, on high grade prostate cancer cells. BMC Medical Genomics. 2009;**2**:55

[51] Wu JB, Lin TP, Gallagher JD, Kushal S, Chung LW, et al. Monoamine oxidase a inhibitor-near-infrared dye conjugate reduces prostate tumor growth. Journal of the American Chemical Society. 2015;**137**:2366-2374

[52] Wang K, Luo J, Yeh S, You B, Meng J, et al. The MAO inhibitors phenelzine and clorgyline revert enzalutamide resistance in castration resistant prostate cancer. Nature Communications. 2020;**11**:2689-2703

[53] Lee HT, Choi MR, Doh MS, Jung KH, Chai YG. Effects of the monoamine oxidase inhibitors pargyline and tranylcypromine on cellular proliferation in human prostate cancer cells. Oncol Reports. 2013;**30**:1587-1592

[54] Zarmouh NO, Messeha SS, Mateeva N, Gangapuram M, Flowers K et al. The antiproliferative effects of flavonoid MAO inhibitors on prostate cancer cells. Molecules 2020; 25: 2257-2286.