Changes in Adhesion-Related Gene Expressions in Microglia under the Influence of Non-Small-Cell Lung Cancer

He B.P.\textsuperscript{i} and Li N.\textsuperscript{ii}

Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore MD10, 4 Medical Drive, Singapore 117597

ABSTRACT

In order to study the interactions between microglial cells (BV2 cells) and Non-Small Cell Lung Cancer (NSCLC) in a tumour-invaded brain parenchyma, \textit{in vitro} co-cultures were established. It facilitates the better understanding of the expression changes of molecules involved in cell adhesion, namely the E-cadherin, E-cadherin associated catenins (α-catenin and β-catenin), E-cadherin binding protein E7 and CD11b/CD18 integrin (also known as Mac-1 complex).

Early researches showed that these molecules are not only involved in cell-cell adhesion, but also play roles in cell migration, cytotoxicity and cell growth. This study investigates the changes in expressions of molecules involved in cell adhesion under the influence of NSCLC cells. In this regard, the mechanisms in which NSCLC employs to evade microglial surveillance, or the methods microglia adopts to carry out its defence against cancerous insults via these molecules are discussed.

The results showed that NSCLC had no significant effects on E-cadherin associated molecules. However, the secreted products of NSCLC were capable of suppressing the expression of CD11b in activated microglia. On the other hand, similar suppressive action was not observed for CD18, the partnering component of CD11b in Mac-1 complex. This suggests that metastatic NSCLC in the brain suppresses microglial activation and Mac-1 up-regulation (a marker of microglia activation) by targeting principally at CD11b expression.

INTRODUCTION

Lung cancer is also the most common cancer cell type that migrates into the brain, accounting for 50-60% of secondary brain tumour cases in the world. Tumour metastasis into the brain poses a huge challenge for modern medicine as almost all cytotoxic chemotherapies, immunotherapies and radiotherapies that work well in controlling systemic metastasis do not perform satisfactorily at tackling tumour problems in the brain. As a result, the risks of relapse among cancer patients increase and survival chances are implicated. Therefore, it is interesting to investigate the ability of the brain’s inherent immune cells, microglial cells, in controlling tumour invasion, in the hope that they can be a target of future cancer drugs in enhancing the success of cancer treatment.

There are many transmembrane molecules present on the plasma membrane of various cell types, including immune cells like microglia and aberrant cells such as tumour. Many of these transmembrane proteins, such as cadherins and integrins, play pivotal roles in modulating cell movement and adhesion, and in the transduction of extracellular signals into intracellular signalling cascades. These signalling pathways can regulate important cellular activities such as cell survival, growth and death. The objective of the study is to find out whether NSCLC can cause a differential expression of various transmembrane molecules, namely E-cadherin, E-cadherin associated catenins (α-catenin and β-catenin), E-cadherin binding protein E7 and CD11b/CD18 integrin. As
such, the influence of NSCLC cells on BV2 cell immune responses mediated by these genes will be discussed.

**METHODS**

Murine microglial BV2 cells and archival NSCLC cells were used in 2 co-culture systems to facilitate the understanding of changes that take place upon their interaction. BV2 cells and NSCLC cells were first labelled with quantum dot nanocrystals that fluoresce at different wavelengths upon excitation. They were then seeded in a chamber on distinct sides and changes between these 2 cells were observed when they migrate and come into proximity with each other. The other co-culture system was established by incubating BV2 cells with serum-free NSCLC-conditioned medium (NSCM) for duration of 48 hours. After the first 24 hours of pre-exposure to NSCM, BV2 cells were activated by 1µg/ml of LPS and incubated for another 24 hours. Control cells were maintained in serum-free RPMI for 48 hours as well. These cells were lysed after the treatment and the RNA was extracted for 2-steps RT-PCR. All experiments were performed in triplicates. Statistical analysis was performed with one-way ANOVA and the level of significance was fixed at $P < 0.05$. The same supernatant co-culture system was applied and the treated BV2 cells were probed with anti-mouse CD11b antibodies for CD11b expression using DAB peroxidise immunocytochemistry technique.

**RESULTS AND DISCUSSIONS**

Figure 1 Confocal microscopy photographs of BV2 cells and NSCLC cells co-culture in chamber. BV2 cells were labelled with red quantum dot nanocrystals while NSCLC cells were labelled with green quantum dot nanocrystals.

The dual culture system comprising of BV2 cells and NSCLC cells showed that when BV2 and NSCLC cells came into proximity (Fig 1.3), there was retraction and shortening of BV2 cytoplasmic protrusions (yellow dashed arrows). BV2 cells became rounded (white arrows) in region with a lot of NSCLC cells. It has been established that BV2 cells change from ramified (as shown in BV2 cells in a region with few NSCLC cells, as pointed by yellow solid arrows) to amoeboid morphology upon activation (Nakajima and Kohsaka, 2001). Therefore, the above result is a tell tale sign that NSCLC cells that metastasize into the brain are capable of activating BV2 cells.
Experimental data showed that the changes in expression of E-cadherin binding protein, E7, α-catenin and β-catenin in BV2 cells were inconsistent when BV2 cells were treated with NSCLC-conditioned medium or activated with LPS. This is probably because E-cadherin associated molecules are not involved in the functions of BV2 cells activated by LPS or tumour.

On the other hand, it was suggested in qRT-PCR results that NSCLC is capable of suppressing Mac-1 through the suppression of CD11b transcription, and not via the suppression of CD18. LPS induced CD11b up-regulation was attenuated in BV2 cells pre-exposed to NSCLC-conditioned medium, whereas the same observation was not made for CD18. The data suggests that CD11b plays a more critical role than CD18 in modulation of Mac-1 expression when BV2 cells encounters NSCLC cells. It is probably because CD11b and CD18 are regulated by different transcriptional pathways. It is therefore speculated that NSCLC cells may be capable of secreting signals that target and prevent increased activity of upstream transcription factor(s) of CD11b, but has not effect on that of CD18 in an event of BV2 activation.

Figure 2 qRT-PCR results showing the differential expression of CD11b (Fig 1A) and CD18 (Fig 1B) when BV2 cells were maintained in RPMI only (control), RPMI+LPS, NSCM or NSCM+LPS for 48 hours. *** refers to P< 0.001. Data is presented in Mean ± Standard Error (S.E.).

Immunocytochemistry of BV2 cells treated with NSCM demonstrated that there is an obvious decrement of CD11b as compared to BV2 cells maintained in serum-free
RPMI. This result suggests that NSCLC can down-regulate CD11b protein expression. It is proposed that NSCLC cells target microglial CD11b via two way suppression. NSCLC cells first suppress the up-regulation of CD11b via transcriptional control. NSCLC then induces a down-regulation of CD11b at translational level, possibly down-regulating the activity of its upstream translational factors. It is speculated that this possibly implicate the assembly of Mac-1 complex, a marker of microglia activation (Herber et. al., 2007).

The suppression of microglia Mac-1 by NSCLC cells may be associated with the inhibition of certain intracellular signalling events that regulate microglia response, such as phagocytosis, adhesion and migration. For instance, reduction of Mac-1 expression may reduce BV2 cells ability to facilitate NSCLC cell death via Fc receptor-mediated antibodies dependent cellular cytotoxicity (Spriel et.al., 2003). Further functional studies that investigate the reasons behind Mac-1 suppression by NSCLC cells may be useful in tackling the problems of treating secondary lung tumours in the brain.

**CONCLUSION**

NSCLC cells are possibly capable of suppressing Mac-1 up-regulation by targeting CD11b. It was suggested by the results that NSCLC cells can offset the effects of LPS on BV2 such that LPS-activated BV2 pre-exposed to NSCLC-conditioned medium did not have an expected up-regulation of CD11b mRNA. NSCLC cells could also further down-regulate microglial BV2 CD11b below baseline at translational level. These suggest that NSCLC cells target microglial BV2 cell CD11b via two way suppression, first by transcriptional control, and then followed by translational control.

Furthermore, NSCLC cells can suppress CD11b but not CD18 mRNA expression in activated microglial BV2 cells. The expression of CD11b and CD18 mRNA are speculated to be controlled by different signalling pathways. It is proposed that NSCLC cells secrete substances that suppress increased activity of signalling pathways unique in mediating CD11b transcription, but not CD18 mRNA expression.

The other genes-of-interest studied, namely the E-cadherin and E-cadherin associated molecules, did not display consistent expressional changes, suggesting that there is no correlation between NSCLC cells and the regulation of these genes in BV2 cells.

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\[\text{Dr He Beiping, Assistant professor, Supervisor of UROPS project}\]

\[\text{Li Na, Student}\]