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Oxytocin receptor pattern of expression in primary lung cancer and in normal human lung

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Summary In order to assess if oxytocin- and vasopressin-induced mitogenic effects detected on small-cell lung carcinoma (SCLC) cell lines could be transposed on primary SCLC, the aim of the present work was to identify mediators of these mitogenic actions on primary tumours samples. This was addressed on normal human lung tissue, on SCLC and on non-SCLC (NSCLC). Herein, we observe, in normal human lung, that OTR is colocalized with vascular endothelial cells of the lung and is not expressed by lung cells of epithelial nature. We detected mRNA amplification of V1aR, V2R and of a V2R variant. We observed that 86% of SCLC biopsies analyzed expressed at least the OTR and that 71% expressed the OTR, the V1aR and the V2R altogether. Comparatively, 50% of NSCLC biopsies tested expressed at least the OTR and 32% expressed the OTR, the V1aR and the V2R altogether. The occurrence of the V1bR/V3R is of 28 and 18% for SCLC and NSCLC, respectively. Nevertheless, for the SCLC biopsies analyzed in this study, V1bR/V3R expression correlates, in all cases, with the expression of all the other neurohypophysial peptide receptors. Our results suggest that neurohypophysial peptide antagonists may offer promise as a potential new therapeutic modality for the treatment of lung cancer expressing at least one of the neurhypophysial peptide receptor subtypes.

Abbreviations: Ab, antibody; CK, cytokeratin; ERK1/2, extracellular signal-regulated kinases 1 ($p44^{MAPK}$) and 2 ($p42^{MAPK}$); MEK1/2, ERK kinases 1 and 2; NSCLC, non-small-cell lung carcinoma; NSE, neuron-specific enolase; OT, oxytocin; OTR, oxytocin receptor; OVTA, $(d(CH_2)_5{}^1,Tyr(Me)^2,Thr^4,Orn^8,Tyr^9\text{-}NH_2)$-vasotocin; PKC, protein kinase C; PLC, phospholipase C; $p90^{RSK}$, p90 ribosomal S6 kinases; RbAb, rabbit polyclonal antibody; SCLC, small-cell lung carcinoma; VP, vasopressin; VPRs, vasopressin receptors; V1aR, V1a vasopressin receptor; V1bR/V3R, V1b/V3 vasopressin receptor; V2R, V2 vasopressin receptor

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1. Introduction

Lung tumours are usually divided in two categories: small-cell lung carcinoma (SCLC), also called 'oat-cell' carcinoma, and non-small-cell lung carcinoma (NSCLC) accounting for 20—25% and 75—80% of cases, respectively. NSCLC are mainly represented by squamous cell carcinoma, adenocarcinoma and large-cell carcinoma [1,2]. SCLC presents an extremely high grade of malignancy with early metastasic invasions. Even though it is primarily highly responsive to chemotherapy, tumour recurrence associated with acquired chemo-resistance leads to an overall cumulative 5-year survival rate of 2% [3,4]. This acquired resistance could lead to the appearance of variant SCLC type [5]. Over the last two decades, despite new drugs development, only minor improvements of survival have been obtained [6,7]. Measured on plasma samples have shown that, in opposition to what was observed with NSCLC, classical SCLC presents neuroendocrine features. Indeed, SCLC epithelial cells are characterized by ectopic secretions of various neuropeptides [8]. In addition, studies on derived SCLC cell lines demonstrated the expression of neuropeptide associated receptors at cell membrane. These combined expressions form autocrine/paracrine loops by which these peptides can modulate oncogenic differentiation and promote tumour growth [9—11]. Among the various neuropeptides acting on SCLC growth, the neurohypophysial antidiuretic hormone, vasopressin (VP), was one of the first to be associated to SCLC ectopic secretions [12—14]. The neoplastic secretion of VP can induce a clinical syndrome characterized by water intoxication, hyponatremia and hypernatriuria known as the Schwartz—Bartter's syndrome [15]. All known VP G-protein-coupled receptor subtypes (VPRs: V1aR, V1bR/V3R and V2R) are expressed by SCLC cell lines [16,17]. Additionally, high concentrations of the other neurohypophysial hormone, oxytocin (OT), are observed in plasma samples of 30—40% of SCLC patients [18—20]. Moreover, the oxytocinergic system, OT and its associated receptor (OTR), was shown to be involved in growth modulation of an increasing number of tumours [21,22]. We demonstrated concomitant OT synthesis and secretion, as well as OTR expression by human SCLC cell lines. OT increases SCLC cell growth, whereas this effect is abolished when cells are incubated with a mix of OT and an OT antagonist $d(CH_2)_5{}^1$,$Tyr(Me)^2$,Thr^4,Orn^8,Tyr^9-NH_2)-vasotocin (OVTA). Additionally, OVTA used alone induces a decrease in SCLC cell proliferation [17]. We also showed that OT- and VP-induced mitogenic effects on SCLC pass through the specific and respective binding of OTR and V1aR and culminate in the activation of ERK1/2 and $p90^{RSK}$ phosphorylation through PLC-, Ca^{2+}-, PKC- and MEK1/2-dependent pathways [23]. These data clearly demonstrate that, in addition to the vasopressinergic system, OT and particularly OTR contribute to give the SCLC tumour a survival advantage. Thus, blocking receptor signalling represents viable pharmacological targets for development of new therapeutic tools for the treatment of tumours expressing neurohypophysial peptide, such as OTR. Concerning the neurohypophysial peptide receptors expression in normal lung, it was demonstrated that the V1aR [24,25] and the V1bR/V3R [26] are expressed by lung of adult rats. V2R mRNA was amplified from lung of adult rats and human [27,28]. Recently, novel sites of non-neoplastic OTR expression were detected [29,30], however, up to now, there is still no data available about OTR expression in human lung.

In order to assess if OT- and VP-induced mitogenic effects detected on SCLC cell lines could be transposed on primary SCLC, the aim of the present work was to identify, on primary tumours samples, the mediators of these mitogenic actions: the neurohypophysial peptide receptors. Moreover, regarding the increasing number of cancer types expressing these receptors and therefore having their growth modulated by exogenous or endogenous neurohypophysial peptide [8,21], we extended our study to NSCLC. In that view, the OTR expression pattern, in comparison with VPRs, was investigated on primary lung tumours samples and this question was addressed on normal human lung tissue as well.

2. Materials and methods

2.1. Reagents

TriPure™ RNA isolation reagent, RNAse-free DNAse I were purchased from Roche (USA). Other reagents were Tissue-Tek® (Sakura, The Netherlands), primers, Moloney Murine Leukemia Virus reverse transciptase, Taq polymerase, ethidium bromide and agarose (Gibco BRL, UK). Glycerol, paraformaldehyde and methanol were obtained from VWR (Belgium). Anti-human OTR rabbit polyclonal antibody (RbAb) [31] was kindly provided by P. Cassoni (University of Torino, Italy), anti-human endothelium mouse monoclonal antibody (mAb) EN4 was purchased from Abcam (UK). Anti-human cytokeratin (CK) mAb MNF 116, anti-human CK RbAb (A575), anti-human neuron-specific enolase