Work in progress report - Thoracic oncologic

OCT4 expression in human non-small cell lung cancer: implications for therapeutic intervention

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Received 18 September 2008; received in revised form 17 November 2008; accepted 19 November 2008

Abstract

Here we investigate the expression of OCT4 human lung adenocarcinoma and bronchioloalveolar carcinoma (BAC) tumor biopsies and tumor-derived primary cell cultures. OCT4 has been detected in several human tumors suggesting a potentially critical role in tumorigenesis. We assessed the presence of OCT4 in clinical tumor samples of both adenocarcinoma and BAC at the cellular and transcriptional levels, respectively. Furthermore, we evaluated tumor-derived cell cultures for potential differences in OCT4 expression. Immunohistochemical analysis depicted OCT4 in 2 of 8 adenocarcinoma tumor samples and 3 of 5 BAC tumor samples, with no apparent difference in the degree of expression among the sections examined. These results were validated by transcript analysis. Flow cytometric assessment of 11 adenocarcinoma-derived cell cultures and 3 BAC-derived cell cultures revealed significantly higher OCT4 expression in adenocarcinoma tumors compared to their normal counterparts. This, however, was not observed in the BAC cultures. Comparative studies of OCT4 in adenocarcinoma and BAC tumor cell cultures demonstrated a dramatically higher expression in the former. The expression of OCT4 may represent a specific and effective target for therapeutic intervention in adenocarcinoma and BAC. In addition, the aberrant expression and distribution of OCT4 may indicate important parameters concerning the differences between adenocarcinoma and BAC.

Keywords: OCT4; Bronchioloalveolar carcinoma; Adenocarcinoma; Cancer stem cells

1. Introduction

Epithelial tumors are by far the most common type of primary malignancies in the lung and constitute the leading cause of cancer morbidity and mortality world-wide [1]. Adenocarcinoma accounts for approximately 33% of lung cancer in women, 15–25% in men, and is defined as a malignant epithelial neoplasm characterized by gland formation [1]. Bronchioloalveolar carcinoma, BAC, is considered a distinctive subtype of pulmonary adenocarcinoma, involving a peripheral tumor with unique clinicopathological features [2]. Although progenitor cells of the bronchiolies (Clara cells) or alveoli (type II pneumocytes) have been implicated, the exact cell source of the oncogenesis has not been determined [3].

Recent reports have demonstrated that adenocarcinoma tumors contain a small subpopulation of cells exhibiting stem cell properties, which have hence been termed cancer stem and/or initiating cells [4, 5]. In addition, it has been suggested that the expression of the molecular marker, Octamer 4, OCT4, plays a crucial role in maintaining these cancer stem cell characteristics and resistive properties [4]. OCT4 belongs to the family of POU-domain transcription factors involved in regulation of cell growth and differentiation. Its expression is normally confined to pluripotent cells of the developing embryo [6]. Strong OCT4 expression is used clinically as a diagnostic tool in primary and metastatic embryonic carcinomas [7]. It has been proposed that OCT4 acts as a multi-functional factor in both cancer and stem cell biology with a possible oncogenic role as well as a key regulator of self-renewal and differentiation, respectively [8].

Although a small number of studies have probed expression of the OCT4 transcript in select human lung cancer tumor samples, to date no one has thoroughly examined the expression of OCT4 protein in non-small cell lung cancer. Here we investigate the expression of OCT4 in human lung adenocarcinoma and BAC tumor biopsies at the cellular and transcriptional levels, respectively. Furthermore, we evaluate tumor-derived cell cultures for potential differences in OCT4 expression.

2. Materials and methods

Methods in detail are available as a supplement from the author.
3. Results

3.1. OCT4 expression in adenocarcinoma and BAC clinical samples

Intra-operative tumor sections which included eight conventional adenocarcinoma samples, and five samples of adenocarcinoma with bronchioloalveolar growth pattern, were investigated for the expression of OCT4 using immunohistochemistry. Our data revealed that sections from five of the thirteen non-small lung cancer tumor specimens depicted positive staining for OCT4. Of these, three were BACs (adenocarcinoma mixed subtypes with bronchioloalveolar growth pattern), and two were adenocarcinomas with no bronchioloalveolar growth pattern (Fig. 1a). Hence positivity was observed in 5 of the 13 samples. Staining of OCT4 was faint in comparison to seminomas which were used as positive controls for OCT4 expression. However, the expression can be clearly detected above the negative controls. Larger sample sizes are currently under investigation to determine the percent positivity in both adenocarcinoma and BAC. In addition, increased sample numbers may potentially indicate differences in the degree and localization of OCT4 between adenocarcinoma and BAC. To validate the immunohistochemical data, we performed OCT4 gene expression analysis on tumor samples obtained immediately after surgical resection. Fig. 1b demonstrates the presence of the OCT4 transcript in clinical biopsies of both adenocarcinoma and BAC.

3.2. Morphology and growth of tumor-derived primary cell cultures

To further investigate the presence of OCT4 at the cellular level, we examined flow cytometric expression of OCT4 in adenocarcinoma and BAC tumor-derived cell cultures. Briefly, we applied a novel cell culture technique, and isolated cells from 14 intra-operative biopsy samples from patients diagnosed with adenocarcinoma (11 samples), BAC (3 samples) (and their respective normal counterparts which served as controls. Primary tumor cell cultures were maintained up to a maximum of 10 days with no passaging to minimize possible modifications resulting from in vitro culture. Cell morphology and growth rate variability were apparent from sample to sample, but cultures from the BAC tumor biopsies generally appeared to grow better in culture and at a faster rate, in comparison to those derived from adenocarcinoma with no bronchioloalveolar pattern. Primary cultures of both tumor sub-types generally appeared as an adherent cell population composed primarily of stromal-like cells, optically translucent flat-adherent, and densely-granulated cuboidal cells. Colony formation was observed as early as three days in culture and was more apparent in the BAC tumor-derived cell cultures. Representative samples of an adenocarcinoma tumor-derived cell culture and BAC tumor-derived cell culture are depicted in Fig. 2a and Fig. 2b, respectively.

3.3. OCT4 expression in primary cultures of BAC and adenocarcinoma

To examine OCT4 expression in the tumor-derived cultures, we used the more sensitive technique of flow cytometry for cellular OCT4 analysis. The primary cultures were expanded and at day 10, were characterized for their expression of OCT4. OCT4 protein was quantified using two different modes of analysis. The first was an assessment based on the mean intensity of OCT4 expression (as emitted by the fluorochrome conjugated antibody) in the cells; and the second was the detection of the number of cells which expressed OCT4 in the corresponding cell culture samples. In agreement with our immunohistochemistry and gene expression data, flow cytometry analyses revealed OCT4 expression in cell cultures derived from both BAC and adenocarcinoma tumors. In addition, flow cytometry illustrated a small percentage of cells with dim expression of OCT4 present in normal samples of both adenocarcinoma and BAC. We then proceeded to assess any differences the OCT4 expression, between the tumor-derived cell cultures and their normal counterparts. There was a significant difference in OCT4 intensity $P=0.036$ (Fig. 3a) as well as...
in the number of cells expressing the protein $P = 0.048$ (Fig. 3b). This, however, held true only for the cells isolated from the adenocarcinoma cell cultures. In contrast to what was observed in adenocarcinomas, there was no difference in either the OCT4 expression intensity or the number of cells expressing OCT4 in the BAC biopsy cell cultures (Fig. 3a,b). Of note, we generally found higher levels of OCT4 expression in the adenocarcinoma tumor-derived cell cultures compared to those derived from BAC tumors (Fig. 4). Whether this alludes to a difference in the degree of OCT4 expression within the NSCLC subtypes remains to be confirmed with the inclusion of larger sample sizes, especially in the case of the BAC-derived cell cultures.

4. Discussion

In this study, we illustrate for the first time, the expression of OCT4 in adenocarcinoma and BAC tissue biopsies as well as in tumor-derived cell cultures. OCT4 immunohistochemistry on tumor tissue sections of BAC and adenocarcinoma illustrated clear staining of OCT4 in 5 of 13 tumor sections analyzed. Although OCT4 staining was depicted in both adenocarcinoma and BAC, immunohistochemistry showed no apparent difference in the degree of expression among the sections analyzed. Genetic expression of OCT4 transcript further validated the histology results and confirmed the presence of OCT4. It is important to note that quantitative real-time PCR on adenocarcinoma and BAC tumor tissue samples is required to elucidate any potential differences in the genetic profiles between the two.

Immunohistochemistry on formalin-fixed paraffin-embedded cell block sections is currently the most widely used ancillary method and has been shown to increase the overall diagnostic accuracy in many studies. However, the use of different protocols, antibodies and clones leads to variable results and limited sensitivity. In contrast, flow cytometry immunophenotyping is reproducible, rapid and has greater sensitivity to detect cellular antigens. Hence, to further examine OCT4 expression at the cellular level, we proceeded to examine tumor-derived cell cultures using flow cytometry. Using a novel cell isolation and culture approach, we successfully cultured and expanded primary cell cultures derived from clinical samples of adenocarcinoma, BAC and their respective normal counterparts. Intriguingly, in contrast to the immunohistochemistry studies, analysis of the tumor-derived cell culture samples portrayed differences in OCT4 expression. We found that primary cell cultures obtained from intra-operative biopsies of adenocarcinoma exhibited significantly higher amounts of OCT4 in comparison to their normal counterparts. This was not true for the BAC cultures, which showed no difference in the number of cells expressing OCT4 and in the intensity of expression when compared to the respective normal cell cultures. Finally, when we compared cell cultures derived from adenocarcinoma tumors to those derived from BAC tumors, we observed significantly higher OCT4 expression in the adenocarcinoma tumor-derived cell cultures.
**Fig. 4.** Difference in OCT4 expression in cells cultured from adenocarcinoma tumor biopsies in comparison to BAC tumor biopsies. (a) Mean OCT4 expression intensity quantified using flow cytometry. (b) Percentage of cells expressing OCT4 quantified by flow cytometry.

Recent reports have demonstrated OCT4 expression in human embryonal carcinomas, testicular germ cell tumors, seminomas, and bladder carcinomas [7, 8]. Its recent appearance in non-germ cell tumors and cancer cell lines implicates OCT4 as an important player as a diagnostic marker in malignancy, and as a potential key factor in carcinogenesis. Clinically, OCT4 has primarily been used as a diagnostic tool [4]. However, recent pre-clinical studies in murine cell lines have demonstrated that forced reduction of OCT4 expression via siRNA technology induces apoptosis of cancer stem cell-like cells. Although this remains to be tested in NSCLC cell lines, the study strongly indicates that targeting OCT4 may have important clinical applications in cancer therapy [10]. The presence of OCT4 in adenocarcinoma and BAC suggests a potential role in the tumorigenesis of the two non-small cell lung carcinomas and mandates further investigation. Our results add to the findings that implicate OCT4 as a multifunctional factor involved in stem cell self-renewal and differentiation as well as carcinogenesis. More specifically, OCT4 can potentially be regarded as a new molecular marker in which its expression might indicate the existence of cancer stem cell-like cells in these tumors.

Understanding lung adenocarcinoma and BAC is a challenge for several reasons. Adenocarcinomas consist of a heterogeneous spectrum of histologic subtypes as well as a wide variety of clinical and radiologic presentations. There are several differences between adenocarcinoma and BAC which indicate the possibility that BAC is a separate pathological entity and, in fact, not a subtype of adenocarcinoma. In addition to morphological and histological differences BAC has a different response to systemic treatments compared with conventional lung adenocarcinoma [11]. Furthermore, the pattern of metastasis and recurrence of BAC differs considerably [4]. Whether in fact BAC is a unique pathological condition separate from adenocarcinoma needs further study. The expression of molecular markers and genetic stamps that could potentially differentiate between the two diseases would be a step towards a better understanding of both non-small cell lung cancer subtypes. Although subsequent studies and quantitative approaches need to be completed to elucidate the importance of OCT4 as a diagnostic marker in lung malignancies, it can potentially be used as a suggestive parameter differentiating between adenocarcinoma and BAC.

Reports have suggested the presence of injury resistant, tumor initiating cells in clinical lung cancer samples as well as in established lung cancer cell lines [12–14]. The recurrent nature of adenocarcinoma and BAC and the modest efficacy of treatment for cancers are concurrent with the cancer stem cell hypothesis and would mean that, like normal stem cells, cancer stem cells are resistant to the cytotoxic effects of chemotherapy and radiotherapy [15]. Curative therapy therefore, may require complete elimination of the cancer stem cell population. Hence, developing strategies to pinpoint these cells will have positive prognostic implications. Whether OCT4 is a marker of a cancer stem cell or tumor initiating cell in adenocarcinoma and BAC cells requires further assessment. However, given its determined role in tumorigenesis and maintenance of cancer stem cell properties, OCT4 expressing cells may represent a specific subset of target cells for effective treatment.

**Acknowledgments**

We would like to thank Dr Lourdes Cortes-Dericks for her technical assistance in cell culture methodology and manuscript editing. We would also like to thank Dr Isabel Breyer and Dr Andy Kappeler for their assistance in genetic analysis and immunohistochemistry, respectively.

**References**


Conference discussion

Dr. S. Elia (Rome, Italy): You said that these were all patients who underwent resection. Did you make any correlation with the stage, and did any of these patients have pleural effusion?

Dr. Karoubi: No. We simply haven’t had enough samples to make any sort of conclusion, and most of these patients were early stages where surgical resection was the only treatment option.

Dr. G.A. Patterson (St. Louis, Missouri, USA): I may have missed it in the presentation. Was there a correlation between OCT4 expression in histologic specimens and the cell cultures from those same tumors? In other words, did all the tumors that expressed OCT4 in histology also have OCT4 in the cell cultures?

Dr. Karoubi: They did, but we find that the cell cultures using flow cytometry is a much more sensitive technique, so there were certain tumors in histology that didn’t express OCT4 but we were able to find OCT4 expression in the cultures derived from them.

Dr. Patterson: And what’s the time course of OCT4 expression in cell culture? Can you take the OCT4 temperature of the culture at various times and get the same result, or is it variable?

Dr. Karoubi: It is variable, but we have done it immediately after we obtained the tumor sample. So the sample is digested, we get the cells and do the OCT4 analysis, and we see similar levels as that which is seen after 10 days in culture, and we culture cells to a maximum of 10 days without any passaging to minimize in-vitro conditioning, but we find that the digestion technique tends to be a little bit harmful to the cells, so we like to let them grow a little bit in culture so that they survive a little bit better.

Dr. Patterson: In your last slide you made this supposition of targeted therapy. First of all, I’m not quite aware what the targeted therapy is for OCT4, but what makes you think that if you blocked OCT4 that there might not be a thousand other markers that would just run around and accomplish exactly the same result in tumor biology?

Dr. Karoubi: Yes, you are absolutely correct, we don’t know that, and this is really just the beginning of looking at cancer stem cells in general. There could be numerous other markers or numerous other compensatory pathways that may result, but it is a beginning.

Dr. T. Chamogeorgakis (Athens, Greece): Do you think you can use target markers to detect earlier occurrence in the future?

Dr. Karoubi: Yes. Again, OCT4 is just one of the markers that has recently been found in literature. Other markers include CD133, something that we’re going to be looking at, and really I think the best is to take the cancer at its earliest stage to look for cancer stem cells.