One-lung Ventilation and HIF1α Expression in Lung Cancer and Pneumothorax

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Abstract. *Background/Aim: A prospective study was designed to investigate the effects of anesthesia, particularly that of the one-lung ventilation procedure (OLV), on the expression of hypoxia-inducible factor 1α (HIF1α) in patients with lung carcinomas and pneumothorax. Materials and Methods: The immunohistochemical expression of HIF1α was studied in formalin-fixed paraffin-embedded tissues from 60 patients who had undergone thoracic surgery for lung cancer (n=48) or pneumothorax (n=12) under OLV general anesthesia. Results: There was a significant, and rather unexpected, association of HIF1α expression with high body mass index (BMI) (p=0.01) and high body weight (p=0.01) of patients with lung carcinomas, but other anesthesia-related parameters, including analysis of arterial oxygen partial tension and anthropometric factors remained insignificant. With regard to pneumothorax cases, these were immunohistochemically unreactive and, hence, no relationship was noted between HIF1α and anesthesia parameters. Conclusion: Anesthesia and OLV procedure performed for lung cancer or pneumothorax does not affect the expression of HIF1α. However, the significant link between high BMI and HIF1α expression noted in patients with lung carcinomas brings forward a possible connection between obesity and hypoxia-related molecular pathways.*

One-lung ventilation (OLV) is a standard practice during thoracic surgery. It improves access to the operation field, isolates and protects the lungs during surgery, and expedites the process of operation (1-3). During OLV, the non ventilated lung collapses but remains perfused, leading to an increase in shunt fraction and impairment of oxygenation (1, 4-7). Oxygen deprivation from ischemia or non-ventilation leads to cellular hypoxic injury. In a recent study, the role of OLV and post-resectional pulmonary oxidative stress in lung cancer patients was assessed (8). It was found that a higher than normal production of oxygen free radicals may possibly cause oxidative damage.

A progressive rise of oxidative stress due to altered reduction–oxidation (redox) homeostasis appears to be one of the hallmarks of the processes that regulate gene transcription in physiology and pathophysiology (9). A crucial transcription factor that is a master regulatory element in sensing hypoxic conditions and in integrating an adapted response *via* gene expression of oxygen-sensitive and redox-sensitive enzymes and cofactors is hypoxia-inducible factor-1 (HIF1) (10, 11).

HIF-1 is a heterodimer of 2 basic helix-loop-helix PAS domain proteins: HIF1α (120 kDa protein) and HIF1β (91-94 kDa protein also called aryl hydrocarbon nuclear receptor translocator, ARNT1 and 2) (12, 13). Increased intracellular content of the HIF-1 occurs immediately following hypoxia sensing. HIF-1α levels are maintained at low levels under normoxic conditions due to continuous degradation *via* the ubiquitin-dependent proteasome pathway (14). Hypoxic stimulation results in rapid increase of HIF1α protein levels, which is not a result of increased mRNA transcription or translation but rather a result of a redox-sensitive stabilization (15). Following HIF1 heterodimerization the complex enters the nucleus and binds to DNA at the hypoxia-response elements (HREs) of target genes. Important genes encoding for erythropoietin (12), vascular endothelial growth factor (VEGF) (16), lactate dehydrogenase (17) and others are under the direct control of HIF1α.

HIF1α protein has been found to increase in a large percentage of human neoplasias, including lung carcinomas (18-21). Although HIF1α overexpression is linked to

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hypoxic tumor environment, oncogenic hypoxia-independent overexpression of the protein through increased translation of the gene has also been documented. Indeed, constitutive expression of HIF1α as a result either of activation of genes, such as protein kinase B *(PKB/Akt)* (22) and epidermal growth factor receptor 2 *(EGFR/HER2)* (23), or even repression of tumor suppressor genes, such as *ING4* (24), may be part of the HIF up-regulatory mechanism. In any case, overexpression of HIF1α has been linked to increased angiogenesis and poor postoperative outcome in patients with non-small cell lung cancer (18-21).

The present study was designed to examine prospectively the effects of anesthesia, OLV and anthropometric variables on the expression of HIF1α in cases of carcinoma and pneumothorax.

Patients and Methods

After obtaining institutional review board approval and informed consent, 60 patients scheduled for elective thoracic surgery were included in this study: 48 for lung cancer, and 12 for pneumothorax. They underwent open thoracotomy requiring OLV under general anesthesia. Of the 48 cases with cancer, 13 were subjected to pneumonectomy and 35 to lobectomy.

Anesthesia was induced with fentanyl (0.25 mg) and etomidate (0.2-0.3 mg/kg) and intubation was facilitated with cisatracurium (0.2-0.3 mg/kg). A double-lumen endobronchial tube (DLT) (Broncho-Cath®; Mallinckrodt Medical, Athlone, Ireland) of an appropriate size was placed for OLV (25). The correct DLT position was confirmed by auscultation and fiberoptic bronchoscopy in the supine, as well as in the lateral decubitus, position (26). Anesthesia was maintained with a continuous infusion of propofol (4-6 mg/kg h) and remifentanyl (0.2-0.3 μg/kg min) as needed. Muscle relaxation was achieved with cisatracurium as required. Ventilatory settings (Julian Ventilator, Draeger®; Luebeck Germany) were identical during 2-lung ventilation (TLV) and OLV and consisted of intermittent positive pressure ventilation with zero applied end-expiratory pressure and peak inspiratory pressure limitation of 35 cmH_2O to prevent barotrauma of the dependent lung. The lungs were ventilated with 100% oxygen. A tidal volume of 8 ml/kg and an inspiratory/expiratory ratio of 1:1 were set. The respiratory frequency (12 to 14 breaths/min) was adjusted to maintain normocarbia ($PaCO_2$ at approximately 40 mmHg).

Hemodynamic and ventilatory variables were monitored throughout the study. Arterial blood gas was drawn before anesthesia induction to determine the PaO_2 under room air breathing (PaO_2 baseline) and after 10 min of TLV in the lateral decubitus position (PaO_2 TLVL). After switching to OLV, arterial blood gas analysis was repeated every 5 min for 30 min and the steady state value of PaO_2 (changes of PaO_2 of less than 7.5 mmHg per minute) was registered (27, 28). The time from anesthesia induction to the surgical excision of the specimen is defined as overall anesthesia time (OA-T) and the time from the onset of OLV in the lateral decubitus position to the surgical excision of the specimen was defined as OLV specimen-time (OLVS-T). The OLVS-T reflects the exposure time of the lung in probable low PaO_2 during OLV to the lateral decubitus position.

The body mass index (BMI) was calculated (www.mydr.com.au/tools/bodymass) and patients were grouped as underweight (BMI<19), normal (BMI 20-25), overweight (BMI 26-30) and obese (BMI>30).

Immunohistochemical technique for HIF1α expression. HIF1α protein was detected using ESEE 122 monoclonal antibody (IgG1 Mab; dilution 1:20) as previously described (29, 30). Formalin-fixed paraffin-embedded sections of 3 μm-thick were deparaffinized and peroxidase was quenched with methanol and H_2O_2 3% for 15 minutes. Microwaving for antigen retrieval was used (4 min). The primary antibody was applied for 90 minutes. Following washing with TBS, sections were incubated with a secondary anti-rabbit anti-mouse antibody (Kwik Biotinylated Secondary, 0.69A Shandon-Upshaw, Pittsburgh, PA, USA) for 15 min and washed in TBS. Kwik Streptavidin peroxidase reagent (039A Shandon-Upshaw) was applied for 15 min and sections were again washed in tris-buffered saline (TBS). The color was developed by 15 min incubation with 3,3' diaminobenzidine (DAB) solution and sections were weakly counterstained with hematoxylin.

Breast cancer tissue sections with strong nuclear HIF-1α expression were used as positive controls. Normal mouse immunoglobulin-G was substituted for primary antibody as the negative control (same concentration as the test antibody).

The percentage of cells expressing HIF1α in the cytoplasm and/or in the nuclei was separately assessed at x200 magnification. Cases were grouped according to the grading system previously reported (31, 32). Briefly, cases with >10% nuclear reactivity and/or strong cytoplasmic expression in >50% of cells were considered as being of high HIF1α reactivity. Lack of any reactivity was considered as negative, while weak cytoplasmic expression (of any extent) or strong cytoplasmic expression in <50% was considered as low HIF1α expression.

Statistical analysis. Statistical analysis and graphic presentation were achieved using the GraphPad Prism 5.0 package (GraphPad, San Diego, CA, USA; www.graphpad.com). Fisher's exact test, chi-squared test or unpaired two-tailed *t*-test was used for testing relationships between categorical variables as appropriate. Linear regression analysis was used to assess the correlation between continuous variables. A Cox proportional hazards model was used to assess the effects of patient and anesthesia variables on HIF1α expression. A *p*-value less than or equal to 0.05 was considered significant.

The median values for the anthropometric and anesthesia variables were used to classify cases into two subgroups (low *vs*. high).

Results

Anthropometric variables. Fifty-five of the patients were male. The median age was 64 years (range 27-82 years). The median weight was 76 kg (range 37-120 Kg) and the median height 170 cm (range 153-189 cm). The median BMI was 25 (range 16-38).

Anesthesia variables. The OA-T and the OLVS-T ranged from 95 to 328 (median 214) minutes and from 34 to 263 (median 149) minutes respectively. The baseline arterial oxygen tension ranged from 58-103 (median 72) mmHg while the PaO_2 value under TLV in the lateral decubitus position and the steady state PaO_2 value under OLV in the same position ranged from 226-628 (median 521) mmHg and from 60-510 (median 147) mmHg respectively. The lung compliance (CL) during OLV ranged from 15 to 47 (median 28) ml/cmH_2O.

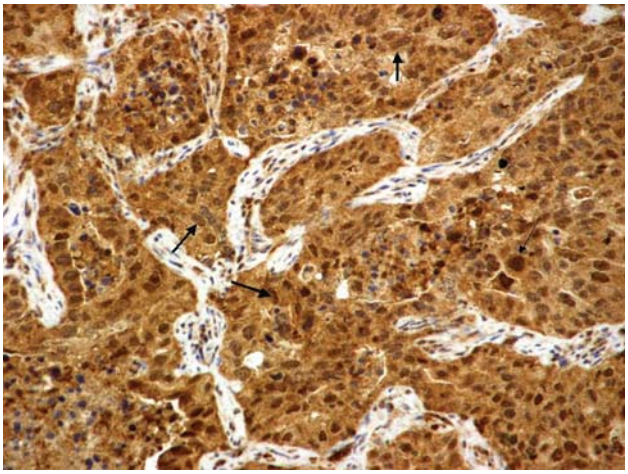

Figure 1. *High cytoplasmic and nuclear HIF1α expression in non-small lung cancer cells, indicated by arrows (immunohistochemical reaction with anti-HIF1α, magnification ×200).*

HIF1a in pneumothorax patients. Anthropometric and anesthesia variables in no way affected the immunohistochemical expression of HIF1α in pneumothorax patients and, indeed, all cases examined were invariably negative for HIF1α expression.

Immunohistochemical HIF1a expression in cancer specimens. Of the 48 cancer cases examined, 23 (47.9%) expressed a low level of HIF1α protein, while 25 (52.1%) showed high HIF1α reactivity (Figure 1). None of the pneumothorax cases expressed high HIF1α reactivity.

Association of HIF-1a with anthropometric variables in cancer patients. A significant association of high BMI (p=0.01) and high body weight with HIF1α (p=0.01), but not of body height, was noted in cancer patients. Age and sex were not related to HIF1α (Table I). In linear regression analysis, the percentage of HIF1α-positive cancer cells was directly related to the BMI (p=0.005, r=0.40) and body weight (p=0.008, r=0.38). Figure 2 shows the distribution of weight (2a) and of BMI (2b) according to HIF1α status.

Association of HIF1a status with anesthesia related variables in cancer patients. Analysis of HIF1α status with regard to anesthesia related parameters, including OA-T, OLVS-T, PaO_2 (baseline, OLV steady-state), and C_L, did not show any significant association either in categorical (Table II) or linear regression analysis.

Association of HIF1a status with type of surgery in cancer patients. The type of surgery (pneumonectomy *vs*. lobectomy) did not affect the expression of HI1α in tumors (p=0.89; data not shown).

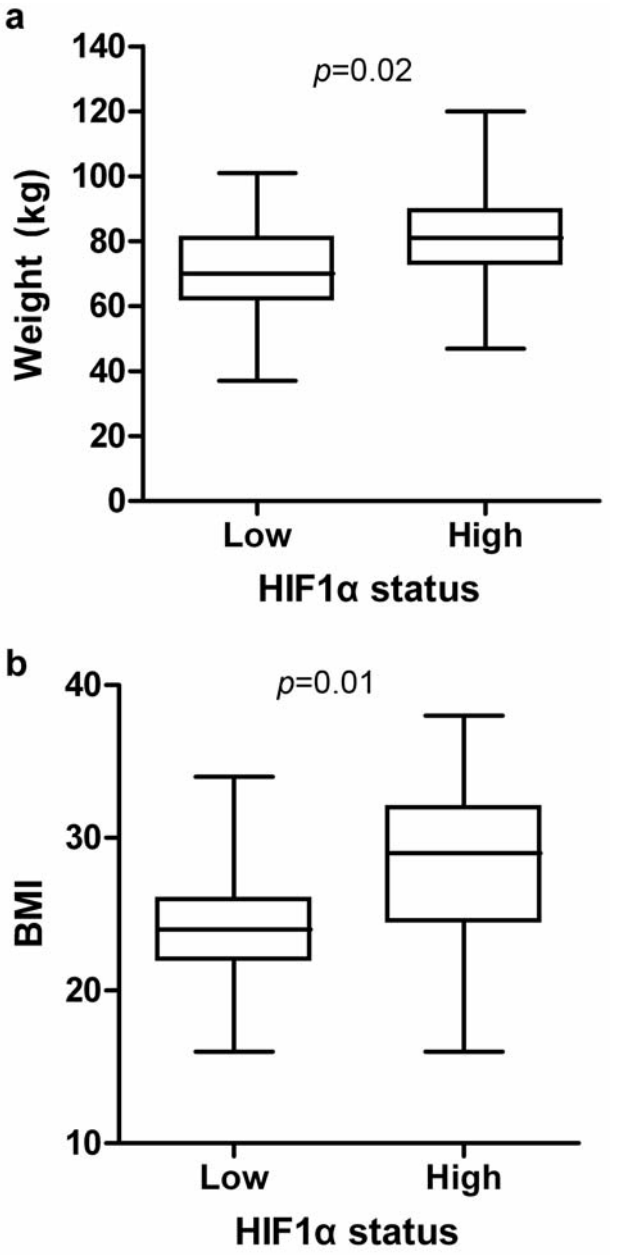

Figure 2. *Body weight (a) and body mass index (b) according to HIF1α status.*

Discussion

Hypoxemia may occur in 5-10% of patients undergoing OLV (3). The fall in PaO_2 occurring during OLV results primarily from the pulmonary shunt of deoxygenated blood through the nonventilated lung and secondarily from ventilation-perfusion mismatch in the ventilated lung (33). Various mechanisms such as hypoxic pulmonary vasoconstriction, gravity and surgical manipulation, cooperate in reducing blood flow and shunting in the nondependent lung and

Table I. *Association of HIF-1α status with anthropometric variables in 48 cancer patients.*

	HIF-1α		
	Low	High	*p*-Value
Gender			
Male	22	23	0.99
Female	1	2	
Age (years)			
≤60	7	8	0.99
>60	16	17	
Height (cm)			
≤170	10	13	0.57
>170	13	12	
Weight (kg)			
≤76	15	7	0.01
>76	8	18	
BMI (kg/m^2)			
Underweight	3	2	
Acceptable	13	6	0.01
Overweight	4	8	
Obese	3	9	

BMI: Body mass index.

Table II. *Association of HIF-1α status with anaesthesia variables in 48 cancer patients.*

	HIF-1α		
	Low	High	*p*-Value
OA-T			
Low	12	12	0.99
High	11	13	
OLVS-T			
Low	12	12	0.99
High	11	13	
PaO_2 baseline			
Low	11	14	0.77
High	12	11	
PaO_2 OLV-steady state			
Low	14	10	0.24
High	9	15	
CL (ml/cmH_2O)			
Low	12	12	0.99
High	11	13	

OA-T: Overall anesthesia time; OLVS-T: one-lung ventilation specimen time, PaO_2: arterial oxygen tension; OLV: one-lung ventilation; CL: lung compliance.

prevent the PaO_2 from decreasing. Despite this, deterioration of oxygenation and occasionally hypoxemia during OLV inducing lung damage is of major concern (1, 4-7, 34, 35). In a model of OLV on Yorkshire pigs by Yin *et al*. (36), it was suggested that lung vascular injury occurs, which was associated with reduced levels of nitric oxide production. Two other studies in animal models showed edema formation and extravascular albumin accumulation after OLV (34, 37).

HIF1α is a major transcription factor regulated by hypoxia (10). Its role in cancer has been extensively studied and overexpression of HIF1α is associated with increased angiogenesis and poor postoperative outcome in patients with non-small cell lung cancer (18-21). Although intratumoral hypoxic conditions or oncogene activation (22-24) may account for up-regulation the HIF1α pathway in a large proportion of non-small cell lung carcinomas, the surgical procedure under OLV may eventually cause deterioration of oxygenation and occasionally hypoxemia, both of which may affect the expression of HIF1α in benign and malignant lung tissues. This would compromise the reliability of a post-surgical assessment of HIF1α expression as a tumor marker in postoperative specimens.

The examination of anthropometric and anesthesia variables during TLV and OLV in parallel with the expression of HIF1α in postoperative tissue specimens revealed that HIF1α was not up-regulated in pneumothorax cases. Hence, these variables do not increase the HIF1α levels in non malignant lung tissues, at least not at a magnitude that could be assessed by immunohistochemical methods.

Similarly, analysis of the OA-T, OLVS-T, PaO_2 (baseline, OLV steady-state), and CL showed lack of any association with the expression level of HIF1α in lung cancer. In accordance with the above findings in non neoplastic pneumothorax lung tissues, the current data suggest that anesthesia and OLV are unlikely to affect HIF1α expression patterns in cancer cells, so that assessment of HIF1α in postoperative lung cancer specimens reliably reflects the preoperative HIF1α status.

It is interesting that obesity and not PaO_2 was significantly related to HIF1α expression in lung carcinomas. In fact, Prado *et al*. directely linked sarcopenic obesity with poor survival in cancer patients (38), implying a connection between obesity and tumor aggressiveness. In colon cancer patients, obesity seems to have an ominous prognostic relevance when tumors overexpress p27 protein (39). Adipose stromal cells in breast tumors stimulate migration and invasion through cytokine production (40), which is known to affect HIF1α expression (41). Increased blood insulin, insulin-like growth factor I, leptin, tumor necrosis factor alpha or interleukin-6 in obese patients may enhance tumor aggressiveness by increasing the phosphoinositide-3-kinase (PI3K) and Akt signal pathway (42). Akt pathway is involved in HIF1α up-regulation in cancer cell lines (43, 44), and this may represent a cross talk between obesity and HIF1α expression.

It is concluded that anesthesia parameters and the OLV procedure performed during thoracic surgery for lung cancer do not affect HIF1α expression as assessed by immuno-

histochemistry. However, the significant link between high BMI and HIF1α expression in lung carcinomas brings forward a possible relation between obesity and hypoxia-related molecular pathways, the pathophysiological and clinical relevance of which demand further investigation.

References

1 Benumof JL: One-lung ventilation and hypoxic pulmonary vasoconstriction: implications for anesthetic management. Anesth Analg *64*: 821-833, 1985.

2 Garutti I, Quintana B, Olmedilla L, Gruz A, Barranco M and de Lucas EG: Arterial Oxygenation during one-lung ventilation: Combined versus general anesthesia. Anesth Analg *88*: 494-499, 1998.

3 Karzai W and Swarzkopf K: Hypoxemia during one-lung ventilation. Prediction, prevention, and treatment. Anesthesiology *110*: 1402-1411, 2009.

4 Schwarzkopf K, Klein U, Schreiber T, Preussler NP and Bloos F, Helfritsch, Sauer F and Karzai W: Oxygenation during one-lung ventilation: The effects of inhaled nitric oxide and increasing levels of inspired fraction of oxygen. Anesth Analg *92*: 842-847, 2001.

5 Watanabe S, Noguchi E, Yamada S, Hamada N and Kano T: Sequential changes of arterial oxygen tension in the supine position during one-lung ventilation. Anesthes Analg *90*: 25-34, 2000.

6 Ishikawa S, Nakazawa K and Makita K: Progressive changes in arterial oxygenation during one-lung anaesthesia are related to the response to compression of the nondependent lung. Br J Anaesth *90*: 21-26, 2002.

7 Sylvester JT: Hypoxic pulmonary vasoconstriction: a radical view. Circ Res *88*: 1228-1230, 2001.

8 Misthos P, Katsaragakis S, Milingos N, Kakaris S, Sepsas E, Athanassiadi K, Theodorou D and Skottis I: Postresectional pulmonary oxidative stress in lung cancer patients. The role of one-lung ventilation. Eur J Cardiothorac Surg *27*: 379-382, 2005.

9 Haddad JJ: Science review: redox and oxygen-sensitive transcription factors in the regulation of oxidant-mediated lung injury: role for hypoxia-inducible factor-1alpha. Crit Care *7*: 47-54, 2003.

10 Semenza GL: HIF-1 and mechanisms of hypoxia sensing. Curr Opin Cell Biol *13*: 167-171, 2001.

11 Semenza GL: HIF-1 and human disease: one highly involved factor. Genes Dev *14*: 1983-1991, 2000.

12 Semenza GL, Nejfelt MK, Chi SM and Antonarakis SE: Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. Proc Natl Acad Sci USA *88*: 5680-5684, 1991.

13 Semenza GL and Wang GL: A nuclear factor induced by hypoxia *via* de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol *12*: 5447-5454, 1992.

14 Huang LE, Arany Z, Livingston DM and Bunn HF: Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. J Biol Chem *271*: 32253-32259, 1996.

15 Huang LE, Gu J, Schau M and Bunn HF: Regulation of hypoxia-inducible factor 1alpha is mediated by an O_2-dependent degradation domain *via* the ubiquitin-proteasome pathway. Proc Natl Acad Sci USA *95*: 7987-7992, 1998.

16 Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD and Semenza GL: Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol *16*: 4604-4613,1996.

17 Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Maire P and Giallongo A: Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. J Biol Chem *271*: 32529-32537, 1996.

18 Giatromanolaki A, Koukourakis MI, Sivridis E, Turley H, Talks K, Pezzella F, Gatter KC and Harris AL: Relation of hypoxia inducible factor 1 alpha and 2 alpha in operable non-small cell lung cancer to angiogenic/molecular profile of tumours and survival. Br J Cancer *85*: 881-890, 2001.

19 Kim SJ, Rabbani ZN, Dewhirst MW, Vujaskovic Z, Vollmer RT, Schreiber EG, Oosterwijk E and Kelley MJ: Expression of HIF-1alpha, CA IX, VEGF, and MMP-9 in surgically resected non-small cell lung cancer. Lung Cancer *49*: 325-335, 2005.

20 Enatsu S, Iwasaki A, Shirakusa T, Hamasaki M, Nabeshima K, Iwasaki H and Kuroki M: Expression of hypoxia-inducible factor-1 alpha and its prognostic significance in small-sized adenocarcinomas of the lung. Eur J Cardiothorac Surg *29*: 891-895, 2006.

21 Yohena T, Yoshino I, Takenaka T, Kameyama T, Ohba T, Kuniyoshi Y and Maehara Y: Up-regulation of hypoxia-inducible factor-1alpha mRNA and its clinical significance in non-small cell lung cancer. J Thorac Oncol *4*: 284-290, 2009.

22 Lentzsch S, Chatterjee M, Gries M, Bommert K, Gollasch H, Dorken B and Bargou RC: PI3-K/AKT/FKHR and MAPK signaling cascades are redundantly stimulated by a variety of cytokines and contribute independently to proliferation and survival of multiple myeloma cells. Leukemia *18*: 1883-1890, 2004.

23 Swinson DE and O'Byrne KJ: Interactions between hypoxia and epidermal growth factor receptor in non-small-cell lung cancer. Clin Lung Cancer *7*: 250-256, 2006.

24 Colla S, Tagliaferri S, Morandi F, Lunghi P, Donofrio G, Martorana D, Mancini C, Lazzaretti M, Mazzera L, Ravanetti L, Bonomini S, Ferrari L, Miranda C, Ladetto M, Neri TM, Neri A, Greco A, Mangoni M, Bonati A, Rizzoli V and Giuliani N: The new tumor-suppressor gene inhibitor of growth family member 4 (ING4) regulates the production of proangiogenic molecules by myeloma cells and suppresses hypoxia-inducible factor-1 alpha (HIF-1alpha) activity: involvement in myeloma-induced angiogenesis. Blood *110*: 4464-4475, 2007.

25 Hannala MS, Benumof JL, McCarthy PO and Liang M: Comparison of three techniques to inflate the bronchial cuff of left polyvinylchloride double-lumen tubes. Anesth Analg *77*: 990-994, 1993.

26 Klein U, Karzai W, Wohlfarth M, Gottschall R and Fritz H: Role of fiberoptic bronchoscopy in conjuction with the use of double-lumen tubes for thoracic anesthesia: a prospective study. Anesthesiology *88*: 346-350, 1998.

27 Moutafis M, Liu N, Dalibon N, Kuhlman G, Ducros L, Castelain MH and Fischler M: The effects of inhaled nitric oxide and its combination with intravenous almitrine on PaO_2 during one-lung ventilation in patients undergoing thoracoscopic procedures. Anesth Analg *85*: 1130-1135, 1997.

28 Aschkenasy SV, Hofer KC, Zalunardo MP, Zaugg M, Weder W, Seifert B, Pasch T and Zollinger A: Patterns of changes in arterial PO2 during one-lung ventilation: A comparison between patients with severe pulmonary emphysema and patients with preserved lung function J Cardiothorac Vasc Anesth *19*: 479-484, 2005.

29 Wiesener MS, Turley H, Allen WE, Willam C, Eckardt KU, Talks KL, Wood SM, Gatter KC, Harris AL, Pugh CW, Ratcliffe PJ and Maxwell PH: Induction of endothelial PAS domain protein-1 by hypoxia: characterization and comparison with hypoxia-inducible factor-1alpha. Blood *92*: 2260-2268, 1998.

30 Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ and Harris AL: The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. Am J Pathol *157*: 411-421, 2000.

31 Koukourakis MI, Giatromanolaki A, Sivridis E, Gatter KC and Harris AL: Tumour Angiogenesis Research Group: Lactate dehydrogenase 5 expression in operable colorectal cancer: strong association with survival and activated vascular endothelial growth factor pathway-a report of the Tumour Angiogenesis Research Group. J Clin Oncol *24*: 4301-4308, 2006.

32 Giatromanolaki A, Koukourakis MI, Turley H, Sivridis E and Harris AL and Gatter KC: Tumour Angiogenesis Research Group: Phosphorylated KDR expression in endometrial cancer cells relates to HIF1alpha/VEGF pathway and unfavourable prognosis. Mod Pathol *19*: 701-707, 2006.

33 Eisekraft JB: Effects of anesthetics on the pulmonary circulation. Br J Anesth *65*: 63-78, 1990.

34 Gama de Abreu M, Heintz M, Heller A, Szechenyi R, Albrecht DM and Koch T: One-lung ventilation with high tidal volumes and zero positive end-expiratory pressure is injurious in the isolated rabbit lung model. Anesth Analg *96*: 220-228, 2003.

35 Michelet P, Roch A, Brousse D, D'Journo XB, Bregeon F, Lambert D, Perrin G, Papazian L, Thomas P, Carpentier JP and Auffray JP: Effects of PEEP on oxygenation and respiratory mechanics during one-lung ventilation. Br J Anaesth *95*: 267-273, 2005.

36 Yin K, Gribbin E, Emanuel S, Orndorff R, Walker J, Weese J and Fallahnejad M: Histochemical alterations in one lung ventilation. J Surg Res *137*: 16-20, 2007.

37 Williams EA, Quinlan GJ, Anning PB, Goldstraw P and Evans TW: Lung injury following pulmonary resection in the isolated, blood-perfused rat lung. Eur Respir J *14*: 745-750, 1999.

38 Prado CM, Lieffers JR, McCargar LJ, Reiman T, Sawyer MB, Martin L and Baracos VE: Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. Lancet Oncol *9*: 629-635, 2008.

39 Ogino S, Shima K, Nosho K, Irahara N, Baba Y, Wolpin BM, Giovannucci EL, Meyerhardt JA and Fuchs CS: A cohort study of p27 localization in colon cancer, body mass index, and patient survival. Cancer Epidemiol Biomarkers Prev *18*: 1849-1858, 2009.

40 Walter M, Liang S, Ghosh S, Hornsby PJ and Li R: Interleukin 6 secreted from adipose stromal cells promotes migration and invasion of breast cancer cells. Oncogene *28*: 2745-2755, 2009.

41 Frede S, Berchner-Pfannschmidt U and Fandrey J: Regulation of hypoxia-inducible factors during inflammation. Methods Enzymol *435*: 405-419, 2007.

42 Huang XF and Chen JZ: Obesity, the PI3K/Akt signal pathway and colon cancer. Obes Rev *10*: 610-616, 2009.

43 Gao N, Shen L, Zhang Z, Leonard SS, He H, Zhang XG, Shi X and Jiang BH: Arsenite induces HIF-1alpha and VEGF through PI3K, Akt and reactive oxygen species in DU145 human prostate carcinoma cells. Mol Cell Biochem *255*: 33-45, 2004.

44 Gort EH, Groot AJ, Derks van de Ven TL, van der Groep P, Verlaan I, van Laar T, van Diest PJ, van der Wall E and Shvarts A: Hypoxia-inducible factor-1alpha expression requires PI 3-kinase activity and correlates with Akt1 phosphorylation in invasive breast carcinomas. Oncogene *25*: 6123-6127, 2006.

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