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# Expression of Erythropoietin and Erythropoietin Receptor in Non–Small Cell Lung Carcinomas

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## ABSTRACT

***Purpose:*** **Expression of erythropoietin (Epo) and its receptor (Epo-R) has been shown in various normal and neoplastic nonhematopoietic tissues. This study, in non–small cell lung carcinoma, was designed to investigate the previously unreported expression of Epo and Epo-R as well as hypoxia-inducible factor-1α (HIF-1α), which is known to control Epo expression.**

***Experimental Design:*** **Samples from lung squamous cell carcinomas (*n* = 17) and adenocarcinomas (*n* = 12) were obtained from patients undergoing curative surgery. mRNA transcripts of Epo, Epo-R, soluble Epo-R (sEpo-R), HIF-1α, and factor inhibiting HIF-1 (FIH-1) were evaluated by reverse transcription-PCR, whereas localization of Epo, Epo-R, and HIF-1α was assessed by immunohistochemistry.**

***Results:*** **Epo, Epo-R, sEpo-R, HIF-1α, and FIH-1 transcripts were detected by reverse transcription-PCR in all samples tested, but with heterogeneous levels of expression for Epo, Epo-R, and sEpo-R. Coordinated levels of mRNA were observed for HIF-1α and FIH-1.**

**Epo was detected in carcinomatous cells by immunohistochemistry in 50% of samples and Epo-R was detected in 96% of samples. Co-expression of Epo and Epo-R was observed on contiguous sections from 50% of tumors. HIF-1α was immunolocalized in 80% of non–small cell lung carcinomas.**

***Conclusion:*** **Epo-R was expressed in almost all samples and Epo was expressed in one half of samples on immunohistochemistry and in 100% of samples by mRNA detection, suggesting a potential paracrine and/or autocrine role of endogenous Epo in non–small cell lung carcinoma. The detection of stabilized HIF-1α suggests a possible role in Epo expression. Moreover, in the light of these results, the potential interactions between therapeutic recombinant Epo and the putative neoplastic Epo/Epo-R signaling pathways must be considered.**

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## INTRODUCTION

Erythropoietin (Epo) is the major cytokine regulating erythropoiesis by binding to the Epo receptor (Epo-R), a member of the cytokine receptor superfamily (1). For many years, Epo was considered to act only on erythroid cells and its synthesis was thought to be limited to the fetal liver and adult kidney (2, 3). Over the last decade, there is growing evidence that many nonhematopoietic organs and tissues express Epo and/or Epo-R (4–7). This widely dispersed Epo/Epo-R expression suggests an autocrine or paracrine role for Epo/Epo-R signaling in addition to erythropoiesis, such as mitogenesis, inhibition of apoptosis, or angiogenesis shown in various cells (8–10).

Co-expression of Epo and Epo-R, shown in various pediatric (11) and adult solid tumors (12–15), also supports the hypothesis that autonomous co-expression could mediate autocrine growth in malignant cells as for erythrocytic leukemia cells (16). The use of recombinant Epo in cancer patients could therefore have potential harmful adverse effects in view of the various effects of Epo on cell proliferation, angiogenesis, and apoptosis inhibition. The widespread use of recombinant Epo in patients with solid tumors should therefore be reappraised by taking into account tumor EpoR expression and its potential adverse effects on tumor control (17, 18).

Only very limited data concerning Epo or Epo-R expression in lung carcinomas have been reported to date. However, this type of study seems to be particularly relevant, as recombinant Epo is widely used during the treatment of non–small cell lung carcinomas (19) and a pioneer study showed binding of biotinylated Epo on sections of human lung carcinomas (20). The present study was therefore designed to evaluate Epo and Epo-R expression on a retrospective series of non–small cell lung carcinomas using real-time reverse transcription-PCR (RT-PCR) for mRNA evaluation and immunohistochemistry for protein localization. We also investigated the Ki67 labeling index to evaluate cell proliferation in relation to Epo/Epo-R expression. Moreover, as the highest levels of Epo and Epo-R expression in breast cancer were shown in hypoxic tumors (21), we concurrently investigated hypoxia-inducible factor-1α (HIF-1α) expression, as, although HIF-1α is not the only hypoxia-induced transcriptional factor, it is one of the most important adaptive responses to hypoxia (22) and Epo is one of its well-documented targets (23). HIF-1 is regulated at two levels: In addition to stabilization of the HIF-1α protein (24), regulation of its transcriptional activity by asparagine hydroxylation, catalyzed by an enzyme called factor inhibiting HIF-1 (FIH-1), is also important (25). We therefore also examined FIH-1 expression in the same samples.

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## MATERIALS AND METHODS

**Tumor Samples.** Non–small cell lung carcinoma samples were collected from patients (25% women; 75% men) undergoing thoracotomy or lobectomy from 2002 to 2003. The study was conducted according to French legislation on biomedical studies. Samples were either stored at −80°C after deep freezing or were paraffin embedded. Twenty-nine non–small cell lung carcinoma samples were investigated: 5 frozen samples of squamous cell carcinomas for mRNA analysis and 24 paraffin-embedded samples for immunohistochemistry. For all non–small cell lung carcinomas (12 squamous cell carcinomas and 12 primary adenocarcinomas) evaluated by immunohistochemistry, resected lung tissues were fixed in 10% buffered formalin (pH 7.0), embedded in paraffin, and 4-μm-thick serial sections were prepared. One of these sections was stained with H&E for histopathologic diagnosis according to the WHO classification (26).

### Evaluation of Epo, Epo-R, sEpo-R, HIF-1α, and FIH-1 Messenger RNA Expression by Real-Time RT-PCR

**RNA isolation.** Total RNA was isolated with TRIzol reagent (Invitrogen Life Technologies, Cergy Pontoise cedex, France) according to the manufacturer's protocol. Briefly, tumor samples were homogenized in TRIzol reagent. After two steps of phenol-chloroform extraction, RNA isolation was done exactly as described in the manufacturer's protocol. The quality and quantity of total extracted RNA samples were then examined using spectrophotometric $A_{260}$ and $A_{280}$ measurements.

**Quantitative Real-Time RT-PCR.** Total RNA (1 μg) from each sample was reverse-transcribed using the Promega RT system (Promega, Charbonnieres, France; reverse transcription: 42°C for 1 h). Two or three microliters of reverse transcription reaction (corresponding to ~25-150 ng of cDNA) were then used for PCR amplification in a 25 μL PCR reaction. Two primers were designed for each gene using Beacon Designer software (Bio-Rad, Marnes-la-Coquette, France). Primer sequences are listed in Table 1 as "F" for forward primers and "R" for reverse primers. Assays were run in duplicate on the iCycler iQ real-time PCR detection system (Bio-Rad). The amplification profile was as follows: Hot Goldstar enzyme activation, 95°C for 3 minutes; 50 cycles of PCR at 95°C, 15 seconds and 60°C, 1 minute. PCR was done according to the manufacturer's protocol using the qPCR Core kit Sybr Green I-No Rox (Eurogentec, Angers, France) with 25 ng of cDNA for actin; 100 ng of cDNA for HIF-1α and FIH-1; and 150 ng of cDNA for Epo, Epo-R, and soluble Epo-R (sEpo-R). The relatively uniform levels of β-actin transcript expression between the various samples in this study allowed the use of β-actin as the standard. The relative level of expression of each gene was therefore computed with respect to the mRNA expression level of the reference β-actin transcript using the following formula: relative mRNA expression = $2^{-(C_t \text{ of gene of interest } - \, C_t \text{ of } \beta\text{-actin})} \times 1{,}000$, where $C_t$ is the threshold cycle value (27). To verify the presence and the predicted size of amplified fragments, PCR products were separated by electrophoresis, visualized in 3% agarose gels with ethidium bromide, and photographed with VersaDoc Imaging system (Bio-Rad).

**Immunohistochemistry for Epo, Epo-R, and HIF-1α Localization.** Four-micrometer-thick serial sections were deparaffinized in xylene and rehydrated in graded alcohols. Slides were steamed in 0.01 mol/L sodium citrate buffer (pH 6.0) for 15 minutes in a microwave oven. After cooling for 20 minutes and washing, specimens were incubated overnight at 4°C with the anti-human Epo rabbit polyclonal antibody (clone H-162; 1/100 dilution corresponding to 2 μg/mL; Santa Cruz Biotechnologies, Inc., Santa Cruz, CA), anti-human Epo-R rabbit polyclonal antibody (clone C-20, 1/400 dilution corresponding to 0.5 μg/mL, Santa Cruz Biotechnologies), and anti-human HIF-1α mouse monoclonal antibody (clone H1α 67-sup, 1/200 dilution corresponding to 15 μg/mL, Abcam Limited, Cambridge, United Kingdom) as previously reported (21, 28). The specificity of these antibodies has been extensively checked by various investigators (21, 28). According to the manufacturer's specifications, anti-human Epo rabbit polyclonal antibody was raised against a recombinant protein corresponding to amino acids 28 to 189, representing mature Epo of human origin, whereas the anti-human Epo-R antibody was raised against a peptide mapping at the carboxyl terminal of Epo-R of human origin. The anti-human HIF-1α mouse monoclonal antibody was generated by a fusion protein containing amino acids 432 to 528 of human HIF-1α.

Sections were then washed with TBS containing 0.1% Tween 20 (pH 7.0) and loaded onto the Ventana IHC Instrument using the Ventana Medical System iView 3,3′-diaminobenzidine detection kit (Ventana Medical Systems, Inc., Tucson, AZ)

*Table 1* List of primers used for real-time RT-PCR

| | Accession number | Sequence | Position | Product length |
|---|---|---|---|---|
| EPO-R | NM_000121 | F: CCTGACGCTCTCCCTCATCC | 892 | 130 |
| | | R: GCCTTCAAACTCGCTCTCTGG | 1,021 | |
| sEPO-R | X57282 | F: TGATTGGCTCAGTTCCACCAG | 66 | 128 |
| | | R:AGGTTGCTCAGCACACACTC | 193 | |
| EPO | NM_000799 | F: GATAAAGCCGTCAGTGGCCTTC | 548 | 76 |
| | | R: GGGAGATGGCTTCCTTCTGGG | 623 | |
| HIF-1α | NM_001530 | F: CCAGCAGACTCAAATACAAGAACC | 2,090 | 138 |
| | | R: TGTATGTGGGTAGGAGATGGAGAT | 2,227 | |
| FIH-1 | NM_017902 | F: TGGCATGAATCCCAGTTGCG | 99 | 112 |
| | | R: GCACCACAGGCTCCTCATTC | 210 | |

Abbreviations: F, forward primers; R, reverse primers.

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according to the manufacturer's recommendations. The sections were counterstained with hematoxylin.

Human fetal liver and placenta sections (2, 7) were used as positive controls for Epo and Epo-R immunolocalization, respectively (data not shown). Negative controls were done for each tumor section, first by omission of the primary antibody and second by incubation with the normal rabbit IgG (dilution 1/200, 2 μg/mL concentration, Santa Cruz Biotechnologies) instead of the primary antibody.

For HIF-1α immunolocalization, laryngeal cancer tissue sections with strong cytoplasmic HIF-1α expression were used as positive controls (data not shown). A mouse monoclonal IgG2b antibody (clone DAK-GO9, DAKO, Trappes cedex, France) was substituted for primary antibody as negative control (dilution 1/580, 15 μg/mL concentration).

All immunohistochemical evaluations were done by three independent observers (K.D., J.F.B., and P.C.) and interobserver variability was minimal. Epo, Epo-R, and HIF-1α expressions were assessed according to the cytoplasmic staining for Epo and Epo-R and cytoplasmic and/or nuclear labeling for HIF-1α.

**Ki67 Labeling Index.** Sections from the same samples were incubated with the monoclonal anti-Ki67 antibody (clone MIB1, dilution 1/150, DAKO) using a similar immunohistochemistry procedure as described above. The Ki67 labeling index was defined as the percentage of Ki67 antigen-expressing cells after counting at least 1,000 tumor nuclei per specimen. The associations between the Ki67 labeling index and the pathologic type of non–small cell lung carcinoma (i.e., squamous cell carcinomas or adenocarcinomas) and with Epo/EpoR expression were analyzed by a Mann-Whitney test, with a limit of significance of $P < 0.05$.

## RESULTS

**Evaluation of Epo, Epo-R, sEpo-R, HIF-1α, and FIH-1 Messenger RNA Expression by Real-Time RT-PCR.** Results are expressed as the mean of duplicate determinations. As shown on Fig. 1, all squamous cell carcinoma samples showed Epo and Epo-R expression. However, this expression was heterogeneous between samples. In addition to the full Epo-R, many Epo-R transcript variants have been described in the literature (29). To investigate whether a previously described potential sEpo-R (30) was also expressed in clinical specimens, we used primers on intron 4 and exon 5 to detect expression of the splice variant due to insertion of intron 4 sequences. The resulting variant encodes for the putative sEpo-R lacking the transmembrane and cytoplasmic domains (30). sEpo-R expression was detected in all samples but with a different pattern from that of Epo-R (Fig. 1*D*). Although HIF-1α expression is mainly regulated at the protein level, we also examined HIF-1α mRNA levels and the levels of the transcriptional regulating enzyme FIH-1. HIF-1α and FIH-1 were both expressed in all tumor samples with a similar pattern of expression (i.e., with a very high level of expression in sample T5; Fig. 1*E* and *F*).

**Immunolocalization of Epo and Epo-R.** Epo expression was detected in tumor cells from 12 non–small cell lung carcinoma samples (50%), more precisely 58% of adenocarcinomas and 42% of squamous cell carcinomas (Table 2). In squamous cell carcinoma, labeling was not homogeneous in tumor lobules, as it was weaker in the inner part of the lobule than in the peripheral cell layers. At the cellular level, the pattern of cytoplasmic labeling was diffuse throughout the cell in squamous cell carcinomas and in adenocarcinomas (Fig. 2*A* and *B*). In adenocarcinomas, when present, intranuclear vacuoles were also stained. By contrast, necrotic areas, present in squamous cell carcinoma, were not stained.

Epo-R localization was revealed by moderate to strong intracytoplasmic granular immunostaining in carcinomatous cells of 23 of 24 non–small cell lung carcinoma. Epo-R was present in carcinomatous lobules of 100% of squamous cell carcinoma and 92% of adenocarcinomas (Table 2). Labeling was homogeneous, except in necrotic areas. At the cellular level, the granular intracytoplasmic labeling was diffusely distributed in the squamous cell carcinoma and reinforced at the apical and basal parts of the adenocarcinoma cells (Fig. 2*C* and *D*). Necrotic or keratinized central areas of squamous cell carcinoma were unlabeled. Labeling was more intense with Epo-R than with Epo (Fig. 2). Contiguous sections of tumor samples showed co-expression of Epo and Epo-R in 50% non–small cell lung carcinoma (i.e., in 58% of adenocarcinomas and 42% of squamous cell carcinomas; Table 2). No staining of carcinomatous cells was observed on any of the tumor sections either after omitting the first antibody or after incubation with normal rabbit IgG (Fig. 2*E* and *F*).

**Immunolocalization of HIF-1α.** HIF-1α expression was detected by immunohistochemistry in 16 of 20 non–small cell lung carcinomas (i.e., in 60% of adenocarcinomas and 100% of squamous cell carcinomas; Table 2). All stainings observed were intracytoplasmic except for one tumor with superadded nuclear staining (Fig. 2*G*). The intensity and extent of staining in the tumor lobules varied from case to case, with heterogeneous staining in individual tumor lobules without a clear peripheral or central pattern. Necrotic or keratinized central areas of squamous cell carcinomas were unlabeled.

When performing negative controls either by omitting the first antibody or after incubation with the normal mouse IgG2b, carcinomatous cells always remained unlabeled (Fig. 2*H*).

**Ki67 Labeling Index.** Tumor cells exhibiting a clear nuclear Ki67 expression were observed on all the sections (data not shown). The percentage of labeled cells was higher in squamous cell carcinoma (mean: 40.4%; range: 16.5-52%) than in adenocarcinomas (mean: 28.8%; range 11-54%; $P < 0.01$). However, no correlation was observed between the Ki67 labeling index and Epo/Epo-R co-expression detected by immunohistochemistry either in squamous cell carcinoma [44.4% in squamous cell carcinoma/Epo+ (range: 35.7-52.21%) and 37% in squamous cell carcinoma/Epo− (range: 16.7-47%; $P = 0.2$)] or in adenocarcinomas [30.4% in adenocarcinomas/Epo+ (range: 11.4-54.1%) and 27% in adenocarcinomas/Epo− (range: 11.1-33.2%; $P = 0.4$)].

## DISCUSSION

Epo-R expression has been shown in several normal tissues with diverse biological effects (5–7). An increasing

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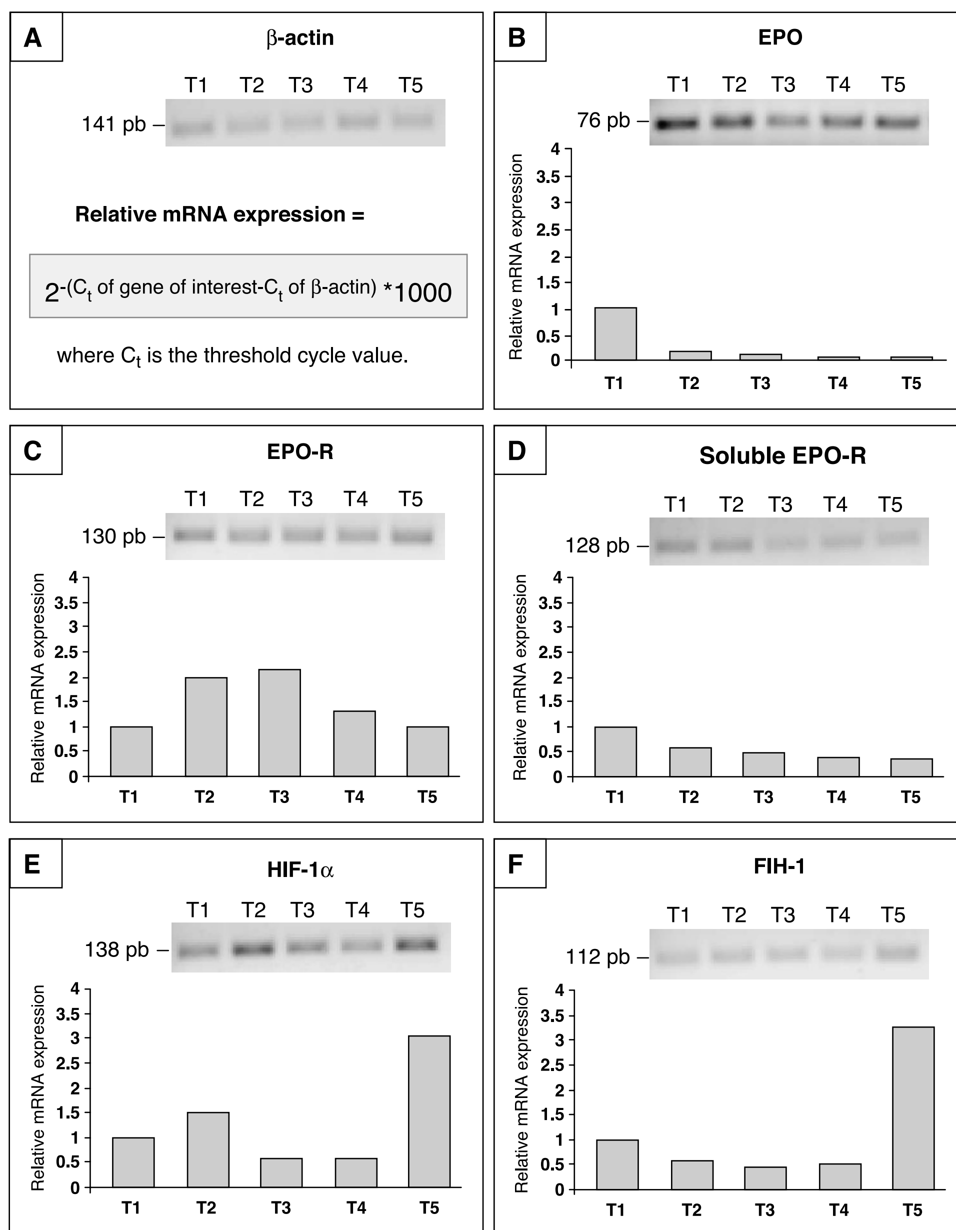


*Fig. 1* Evaluation of the expression of Epo (*B*), Epo-R (*C*), sEpo-R (*D*), HIF-1α (*E*), and FIH-1 (*F*) mRNA from five non–small cell lung carcinoma samples using quantitative real-time RT-PCR. The level of expression was determined relative to the standard β-actin transcript (*A*) using the formula indicated and was then compared with the level of expression of sample 1, which is equal to 1.

number of reports dealing with Epo and Epo-R expression in tumors or cancer cell lines also suggest a significant role of Epo/Epo-R signaling in tumors (11–15). For instance, it has recently been clearly shown that Epo induces an anti-apoptotic response in tumor cells, as well as an angiogenic response with vascular endothelial growth factor release from tumor cells (11). In this study, we report that co-expression of Epo and its receptor at the mRNA and protein level is a common finding in non–small cell lung carcinoma regardless of the subtype: squamous cell carcinoma or adenocarcinoma. No

*Table 2* Results of immunohistochemistry on non–small cell lung carcinoma tissue sections expressed as the number of tumors labeled after incubation with anti-Epo, anti-Epo-R, and anti-HIF-1α antibodies

| | Epo | Epo-R | Epo/Epo-R | HIF-1α | HIF1α/Epo |
|---|---|---|---|---|---|
| Squamous cell carcinomas | 5/12 (42%) | 12/12 (100%) | 5/12 (42%) | 10/10 (100%) | 5/10 (50%) |
| Adenocarcinomas | 7/12 (58%) | 11/12 (92%) | 7/12 (58%) | 6/10 (60%) | 4/10 (40%) |
| Total | 12/24 (50%) | 23/24 (96%) | 12/24 (50%) | 16/20 (80%) | 9/20 (45%) |

NOTE. Co-expressions of Epo and Epo-R as well as Epo and HIF-1α evaluated on serial sections are also indicated.

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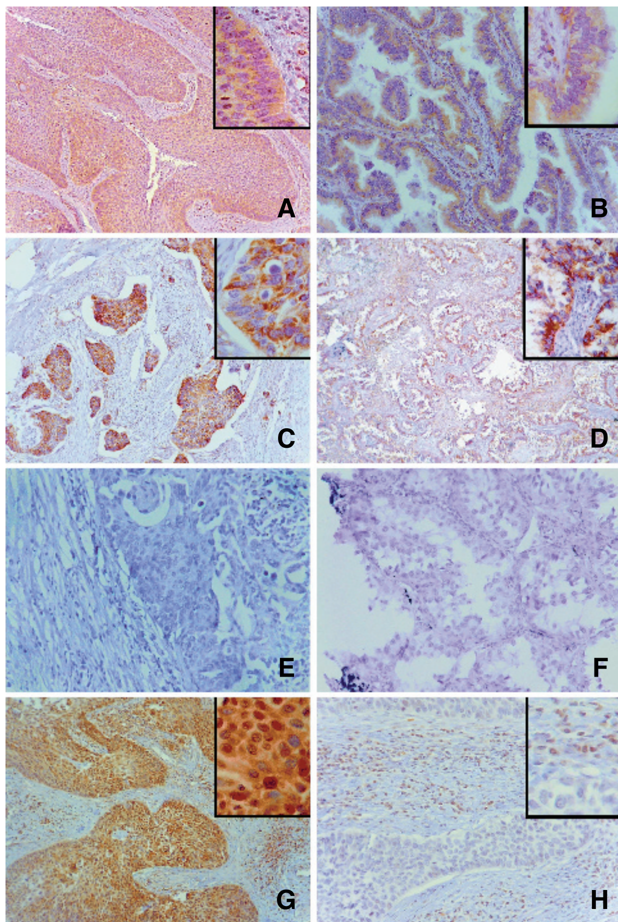


*Fig. 2* Immunoperoxidase staining of Epo (*A* and *B*) and Epo-R (*C* and *D*) on serial sections from squamous cell carcinomas (*A*, *C*, *E*, and *G*) and adenocarcinomas (*B*, *D*, *F*, and *H*). *A*, squamous cell carcinoma section incubated with anti-human Epo rabbit polyclonal antibody. Labeling is more intense at the periphery of the tumor lobules (magnification, ×80). *Inset,* diffuse intracytoplasmic labeling (magnification, ×300). *B,* adenocarcinoma section (papillary subtype) incubated with anti-human Epo rabbit polyclonal antibody showing homogeneous labeling of carcinomatous cells (magnification, ×100). *Inset,* diffuse intracytoplasmic labeling (magnification, ×300). *C,* squamous cell carcinoma section incubated with anti-human Epo-R rabbit polyclonal antibody. Labeling is homogenously observed throughout the carcinomatous lobules (magnification, ×100). *Inset,* granular intracytoplasmic labeling (magnification, ×300). *D,* adenocarcinoma section (a composite subtype) incubated with anti-human Epo-R rabbit polyclonal antibody (magnification, ×100). *Inset,* granular intracytoplasmic labeling (magnification, ×300). *E,* negative control for Epo and Epo-R immunohistochemistry; squamous cell carcinoma section incubated with the nonimmune rabbit Ig (magnification, ×150). *F,* negative control for Epo and Epo-R immunohistochemistry; adenocarcinoma section (papillary subtype) incubated with nonimmune rabbit Ig (magnification, ×150). *G,* squamous cell carcinoma section incubated with anti-human HIF-1α mouse monoclonal antibody. Tumor lobules are homogeneously labeled (magnification, ×100). *Inset,* nuclear and cytoplasmic staining (magnification, ×300). *H,* negative control for HIF-1α immunohistochemistry; squamous cell carcinoma section incubated with nonimmune mouse IgG2b (magnification, ×180). *Inset,* nonspecific staining of stromal cells (magnification, ×300).

previous reports of Epo or Epo-R expression in non–small cell lung carcinoma have been published apart from a pioneer study done with recombinant glycosylated biotinylated Epo, which revealed binding sites with a specificity for Epo in lung cancer (20).

Epo and Epo-R co-expression were detected in all samples when tested by RT-PCR, whereas immunohistochemistry localized Epo in 50% of samples and Epo-R in 96% of samples (100% of squamous cell carcinoma and 92% of adenocarcinoma). These results agree with those previously reported in other solid tumors (e.g., in breast carcinomas tumors; ref. 13) and in a wide range of pediatric tumors where Epo and Epo-R expression was detected more frequently by RT-PCR than by immunohistochemistry (11). It has been suggested that Epo-R expression in tumors could be induced by an oncogenic mechanism (15) and could be related to cell-cycle events with a higher expression observed on cells undergoing active cell division (31). The up-regulation of functional Epo-R along with Epo may contribute to the selection of cells with diminished apoptotic potential (11, 15, 32) and relative resistance to therapy (33).

In addition to the full-length form of Epo-R, many Epo-R transcript splice variants have been discovered, corresponding to insertions from introns, unspliced introns, or skipped exons (29, 30), resulting, for example, in cytoplasmic and/or membrane truncated and/or sEpo-R. Using different sets of primers for Epo-R mRNA evaluation, we detected transcripts for membrane-bound Epo-R and for sEpo-R. Different isoforms of Epo-R have been recently reported in cancer cell lines and in primary tissues, particularly in a purchased sample of mRNA of lung cancer biopsies (29). The presence of sEpo-R mRNA, which needs to be confirmed by protein evaluation, could modulate Epo/Epo-R signaling in non–small cell lung carcinoma. The presence of soluble receptors for various cytokines has been shown to either antagonize or prolong the half-life of these mediators (34, 35). Therefore, sEpo-R expression in cancer cells may modulate the effects of either exogenous or local Epo. However, sEpo-R mRNA expression in lung cancers must be investigated, as a previous study failed to detect sEpo-R protein in the conditioned growth medium of lung cancer cells (36).

Epo and Epo-R co-expression in non–small cell lung carcinoma suggests that an autocrine or paracrine mechanism of Epo signaling may also play a role in non–small cell lung carcinoma. As reported by others (37), we observed a significant difference in the Ki67 labeling index between squamous cell carcinoma and adenocarcinomas; however, in this limited series of patients, we did not find any relationship between Ki67 labeling index and Epo/Epo-R co-expression. This result could be at least partially explained by the shown association between Ki67 labeling index in non–small cell lung carcinoma and multiple clinicopathologic factors not evaluated in the present series (37).

On the other hand, Epo/Epo-R signaling in tumor cells has been shown to clearly act via inhibition of apoptosis, which was also not evaluated in the present study (32). The role of Epo/Epo-R signaling in non–small cell lung carcinoma therefore needs to be tested on cell lines, as reported for other cancer cell types (38–40).

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Extrarenal Epo expression in neoplastic tissues (11–15) and in normal tissues (2, 4, 5, 7) has also been shown to be largely controlled by oxygen-dependent pathways (24). Hypoxia has also been recently shown to induce Epo-R expression in cancer cell lines (21). Most of the genes activated during hypoxia are regulated by HIF-1α or its close relatives HIF-2α or HIF-3α (23, 41, 42). Although transcriptional expression of HIF-1α has been reported to be amplified during hypoxia, the primary mechanism of regulation has been shown to be posttranslational (43). HIF-1α is therefore rapidly degraded under normoxic conditions. The HIF transcription factor then activates the transcription of >60 hypoxia-inducible genes, including Epo, proteins controlling cell proliferation, drug resistance, such as MDR1, or angiogenesis (44). It can be hypothesized that Epo expression in non–small cell lung carcinoma as well as in other solid tumors could be related to intratumoral hypoxia, which is a constant feature of solid tumors (45, 46). HIF-1α expression has been documented in non–small cell lung carcinomas (47, 48) and has been found to be associated with improved as well as poorer prognosis (48, 49). However, it has recently been clearly shown that a high level of HIF-1α expression is associated with a poor prognosis (47). In the present study, we detected HIF-1α expression at the mRNA and protein levels by immunochemistry in 80% of samples. Different molecular events regulating HIF-1α expression in adenocarcinoma and squamous cell carcinoma can be hypothesized, as HIF-1α was detected in 100% of squamous cell carcinoma compared with 60% of adenocarcinomas. However, the labeling pattern was cytoplasmic except in one squamous cell carcinoma sample with superadded nuclear staining. In various studies, HIF-1α and HIF-2α were both detected in the cytoplasm and in the nucleus, and the authors regarded both expression patterns as positive labeling (48). One explanation for cytoplasmic labeling could be related to the presence of FIH-1 detected at the mRNA level in our samples. FIH-1 is a unique asparaginyl-hydroxylase mainly located in the cytoplasm interacting with HIF-1α, which could therefore maintain HIF-1α within the cytoplasm (25). Interestingly, in this study, we found that HIF-1α and FIH-1 were expressed in all tumor samples with a similar profile. For example, particularly high expression of both HIF-1α and FIH-1 was detected in sample T5. This suggests a putative negative feedback of HIF-1 expression, as reported for the expression of hypoxia-induced HIF-1 prolyl hydroxylases (50, 51). It can be hypothesized that the presence of FIH-1, not yet shown in non–small cell lung carcinoma, could therefore modulate the role of HIF-1α in such tumors.

In conclusion, in this study of a series of non–small cell lung carcinoma, Epo-R expression was shown in almost all samples studied. Epo expression was also detected in one half of samples, suggesting a potential paracrine and/or autocrine role of endogenous Epo in non–small cell lung carcinomas. Although recombinant human Epo is of value to treat anemia in cancer patients, particularly as an adjunct to chemotherapy (52), the harmful consequences of Epo related to cancer cell survival (11), proliferation (38), angiogenesis, and promotion of tumor growth (39) challenge its use in solid tumors (17, 53). Our findings, showing that Epo-R is expressed in almost all non–small cell lung carcinomas, raise the question of their potential activation by recombinant human Epo, which needs to be investigated experimentally particularly in transplanted models of human non–small cell lung carcinoma, as previously recommended (39). Epo/Epo-R interactions have also been shown in various cell lines to be responsible for increased resistance to cisplatin, which is one of the major drugs used in non–small cell lung carcinoma (54). Epo and Epo-R expression in non–small cell lung carcinoma therefore need to be tested as novel prognostic biological parameters in a large series of stage I to II patients.

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