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Mieczyslaw Pokorski *Editor*

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Mieczyslaw Pokorski
Editor

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Editor
Mieczyslaw Pokorski
Public Higher Medical Professional School in Opole
Institute of Nursing
Opole, Poland

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Preface

The book series *Neuroscience and Respiration* presents contributions by expert researchers and clinicians in the field of pulmonary disorders. The chapters provide timely overviews of contentious issues or recent advances in the diagnosis, classification, and treatment of the entire range of pulmonary disorders, both acute and chronic. The texts are thought as a merger of basic and clinical research dealing with respiratory medicine, neural and chemical regulation of respiration, and the interactive relationship between respiration and other neurobiological systems such as cardiovascular function or the mind-to-body connection. The authors focus on the leading-edge therapeutic concepts, methodologies, and innovative treatments. Pharmacotherapy is always in the focus of respiratory research. The action and pharmacology of existing drugs and the development and evaluation of new agents are the heady area of research. Practical, data-driven options to manage patients will be considered. New research is presented regarding older drugs, performed from a modern perspective or from a different pharmacotherapeutic angle. The introduction of new drugs and treatment approaches in both adults and children also is discussed.

Lung ventilation is ultimately driven by the brain. However, neuropsychological aspects of respiratory disorders are still mostly a matter of conjecture. After decades of misunderstanding and neglect, emotions have been rediscovered as a powerful modifier or even the probable cause of various somatic disorders. Today, the link between stress and respiratory health is undeniable. Scientists accept a powerful psychological connection that can directly affect our quality of life and health span. Psychological approaches, by decreasing stress, can play a major role in the development and therapy of respiratory diseases.

Neuromolecular aspects relating to gene polymorphism and epigenesis, involving both heritable changes in the nucleotide sequence and functionally relevant changes to the genome that do not involve a change in the nucleotide sequence, leading to respiratory disorders will also be tackled. Clinical advances stemming from molecular and biochemical research are but possible if the research findings are translated into diagnostic tools, therapeutic procedures, and education, effectively reaching physicians and patients. All that cannot be achieved without a multidisciplinary, collaborative, bench-to-bedside approach involving both researchers and clinicians.

The societal and economic burden of respiratory ailments has been on the rise worldwide leading to disabilities and shortening of life span. COPD alone causes more than three million deaths globally each year. Concerted efforts are required to improve this situation, and part of those efforts are gaining insights into the underlying mechanisms of disease and staying abreast with the latest developments in diagnosis and treatment regimens. It is hoped that the books published in this series will assume a leading role in the field of respiratory medicine and research and will become a source of reference and inspiration for future research ideas.

I would like to express my deep gratitude to Mr. Martijn Roelandse and Ms. Tanja Koppejan from Springer's Life Sciences Department for their genuine interest in making this scientific endeavor come through and in the expert management of the production of this novel book series.

Opole, Poland Mieczyslaw Pokorski

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The Role of Dysregulated MicroRNA Expression in Lung Cancer

M. Krutakova, M. Sarlinova, T. Matakova, A. Dzian, J. Hamzik, M. Pec, S. Javorkova, and E. Halasova

Abstract

MicroRNAs (miRNAs) are a class of small single-stranded non-protein-coding RNAs that play important regulatory roles in many cellular processes including cell proliferation, differentiation, growth control, and apoptosis. They regulate gene expression on the posttranscriptional level by translational repression, mRNA cleavage, or mRNA degradation in various physiological and pathological processes. In addition, some miRNAs can function as oncogenes or tumor suppressors, so they can regulate several genes that play important roles in tumorigenesis. It was found that miRNAs are directly involved in many types of cancer, including lung cancer. Lung cancer is the leading cause of cancer mortality worldwide with a substantially low survival rate. In this work, we summarize recent findings related to miRNAs mechanisms of action and the role of their dysregulated expression in lung tumorigenesis. We describe the most important miRNAs involved in lung cancer development and targets of their activity. The understanding of the miRNA regulation in cancer may help better understand the molecular mechanisms of tumorigenesis and their importance in cancerous transformation.

M. Krutakova and M. Sarlinova
Division of Molecular Medicine, Biomedical Center Martin, Comenius University in Bratislava, Mala Hora 4/C, 03601 Martin, Slovakia

T. Matakova
Department of Medical Biochemistry, Comenius University in Bratislava, Mala Hora 4/D, 03601 Martin, Slovakia

A. Dzian and J. Hamzik
Clinic of Thoracic Surgery and University Hospital Martin, Comenius University in Bratislava, Kollárova 2, 03601 Martin, Slovakia

M. Pec
Department of Medical Biology, Comenius University in Bratislava, Mala Hora 4/A, 03601 Martin, Slovakia

S. Javorkova
Clinic of Pediatrics, Central Military Hospital, Generala Milosa Vesela 21, 03426 Ruzomberok, Slovakia

E. Halasova (✉)
Division of Molecular Medicine, Biomedical Center Martin, Comenius University in Bratislava, Mala Hora 4/C, 03601 Martin, Slovakia

Department of Medical Biology, Comenius University in Bratislava, Mala Hora 4/A, 03601 Martin, Slovakia
e-mail: halasova@jfmed.uniba.sk

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1 Introduction

Cancer is one of the most serious diseases around the world. There were estimated 14.1 million cancer cases in 2012 and this number is expected to increase to 24 million by the year 2035. Lung cancer is the most common cancer and the leading cause of cancer related mortality in many economically developed countries. In 2012, lung cancer contributed to 13 % of newly diagnosed cases. Smoking contributes to 85 % of lung cancers (Ferlay et al. 2013). It accounts for approximately 1.6 million deaths each year. There are two main histological types of lung cancer: non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). The majority of all cases are classified as NSCLC (85 %) and 15 % of cases as SCLC. There are three main subtypes of NSCLC, which are adenocarcinoma (approx. 40 % of cases), squamos-cell lung carcinoma (25–30 % of cases), and large cell lung carcinoma (10–15 % of cases) (Oyewumi et al. 2014; Siegel et al. 2014). The poor prognosis of lung cancer is due to late diagnosis, tumor heterogeneities within histological subtypes and limited understanding of tumor biology (Lin et al. 2010).

Cancerogenesis is a long multi-step process. There are five major steps for cancer development: initiation, promotion, malignant transformation, progression, and metastasis (Zhang et al. 2007). These changes are caused by carcinogens, mutagens, defect repair mechanisms, and also by the action of some epigenetic factors. The crucial steps of healthy tissue maintenance among others are regulated gene expression, posttranslational modifications of proteins, molecular interactions, signaling pathways with feedback, repair of DNA damage and the removal of damaged cells by apoptosis. This regulation is organized by, e.g., functional growth factors, growth factor receptors, signal transducers, transcription factors, and other regulatory proteins controlling cell cycle. Deregulation of these processes can lead to malignant transformation and tumor formation (Zmetakova et al. 2013; Adamkov et al. 2012). The formation of cancer is also influenced by the combined interaction of two factors – tumor suppressors, which inhibit cancer development and cancer inducers, oncogenes, which promote cancer development (Zhang et al. 2007). Oncogenes and tumor suppressors are often involved in the molecular changes and these alterations lead to deregulation of key cell regulatory and growth control pathways (Mitsuuchi and Testa 2002).

Tumorigenesis is also regulated by small non-coding RNAs, which are key players in the development of cancer (Wu et al. 2015). MicroRNAs (miRNAs) are small single-stranded, non-protein-coding RNA molecules with the length of 19–25 nucleotides (Bartel 2004). miRNAs regulate gene expression on the posttranscriptional level through interaction with 3′ untranslated regions of mRNAs (Yekta et al. 2004). They play important roles in many cellular processes including cell proliferation, differentiation, growth control, and apoptosis (Medina and Slack 2008). Mature miRNAs and Argonaute proteins form the RISC (RNA-induced silencing complex) that can mediate gene silencing through induction of mRNA cleavage, degradation, or translation repression (Liu et al. 2004; Pillai et al. 2004). By regulating gene expression at the posttranscriptional level, miRNAs can influence various physiological and pathological processes in cells (Naidu and Garofalo 2015). Each miRNA can target several different mRNAs and conversely, a single mRNA can be targeted by several miRNAs (Melo and Esteller 2011). In addition, several miRNAs can function as oncogenes – promote

cancer development, or tumor suppression – inhibit tumor development, so they have a great impact on developmental and oncogenic pathways (Zhang et al. 2007). Expression of oncogenic miRNAs is increased in cancer, they can stimulate cancer development and inhibit the translation of tumor suppressor genes (Kumar et al. 2007). Conversely, expression of tumor suppressor miRNAs is reduced in cancer (Fortunato et al. 2014).

A better understanding of the molecular and cellular biology of lung cancer, defining the common molecular pathways and the role of miRNAs in the regulation of these processes may identify new biomarkers and screening tests and find more effective therapeutic strategies (Bianchi 2015; Wang et al. 2015). In this review, we describe the most important miRNAs operating in lung cancer, their targets and mechanisms involved in lung cancer development. We summarize recent findings related to the role of dysregulated expression in lung carcinogenesis.

2 Dysregulated MicroRNA Expression in Lung Cancer

miRNAs are directly involved in development of many types of cancer. Numerous studies have reported findings of dysregulated expression of miRNA in lung cancer patients. Several miRNAs are dysregulated in lung cancer and have been documented to have tumor-promoting or tumor-suppressing effect in cell cycle regulation, programmed cell death, invasion and metastasis, or in angiogenesis (Joshi et al. 2014). An understanding of miRNA regulation is essential for gaining insight into in lung tumorigenesis.

2.1 Epidermal Growth Factor Receptor

One of the main characteristics of cancerogenesis is deregulated cell cycle. Alterations of the epidermal growth factor receptor (EGFR) are involved in the pathogenesis of NSCLC (Gasparini et al. 2015). The EGFR family of transmembrane tyrosine kinase receptors, also called HER/EGFR/ERB, includes four members – EGFR, Erb-2 (HER-2), Erb-3 and Erb-4 (Normanno et al. 2005). They are involved in the regulation of numerous oncogenic functions, cell proliferation, differentiation, survival, neovascularisation, invasion, and metastasis (Sordella et al. 2004; Yarden and Sliwkowski. 2001). Mutations in EGFR lead to constitutive tyrosine kinase activation and oncogenic transformation of lung cells (Cooper et al. 2013; Greulich et al. 2005). Dysregulated miRNA expression in the cell contributes to disruption of EGFR signaling pathway. Chan et al. (2012) predicted 138 miRNAs that could potentially target EGFR signaling pathway in NSCLC. For example, miR-128 directly controls the signaling pathway of EGFR in lung cancer (Weiss et al. 2008). miR-128 loss of heterozygosity is frequently found in NSCLC patients and is positively correlated with survival. Hu et al.(2014) reported that the expression level of miR-128 is significantly downregulated in NSCLC cancer cells and cancer tissues. The overexpression of miR-128 suppresses proliferation, migration, invasion of cancer cells, and induces G1 arrest and apoptosis in NSCLC cells. In addition, overexpression of miR-128 directly targets VEGF-C (vascular endothelial growth factor receptor), so that miR-128 plays a role in modulation of angiogenesis and lymphangiogenesis.

The expression of miRNAs correlates with that of EGFR and with EGFR mutational status or signaling activities. Thus, miRNAs emerge as unique effectors of the EGFR signaling pathway (Bjaanaes et al. 2014). Dacic et al. (2010) reported a correlation of miRNA expression in lung adenocarcinomas with different oncogenic mutations, including EGFR-positive mutation, KRAS-positive mutation, and EGFR/KRAS-negative tumors. There is overexpression of miR-155 in EGFR/KRAS-negative samples. miR-25 is upregulated only in the EGFR-positive group and miR-495 is upregulated only in KRAS-positive adenocarcinomas. Conversely, in EGFR/KRAS-negative adenocarcinomas the let-7 g is downregulated. These results show

that some miRNAs are in strong correlation with the mutation type of tumors.

miR-7 is frequently downregulated in lung cancer. It negatively regulates the EGFR signaling pathway at multiple levels. miR-7 downregulates the expression of EGFR and murine leukemia viral oncogene homolog-1 *RAF-1* oncogenes (Li et al. 2014). The effect of miR-7 action is to inhibit cell cycle progression and reduce cell growth (Webster et al. 2009). In addition, Xiong et al. (2011) reported that overexpression of miR-7 suppresses cell proliferation, induces cell apoptosis, and inhibites cell migration *in vitro*, and also reduces tumorigenecity *in vivo*. These authors suggest that miR-7 regulates the expression of *BCL-2* through direct 3′UTR interactions.

Wang et al. (2014) showed that the expression of miR-133a negatively correlates with cell invasion, metastasis, and proliferation of lung cancer cell lines by inhibiting EGFR, and transforming growth factor-beta receptor TGFBR and insulin-like growth factor 1 receptor IGF-1R.

A crucial miRNA for cell proliferation in NSCLC cells is the miR-145. Guo et al. (2014) showed that the EGFR signaling pathway mediates the downregulation of miR-145 through ERK1/2 signaling molecules in lung cancer cells. Cho et al. (2011) demonstrated that the mRNA expression of EGFR and *NUDT1* (8-oxo-dGTPase) were significantly downregulated after miR-145 transfection in human lung adenocarcinoma cells. *NUDT1* (8-oxo-dGTPase) is involved in accumulated mis-incorporation of 8-oxo-dGTP into DNA, which can lead to dysfunction and cell death (Sakumi et al. 1993).

2.2 RAS/RAF/MEK/MAPK Pathway

Other miRNAs that are involved in the regulation of gene expression in lung cancer cells are grouped into the let-7 family of miRNAs (Shin et al. 2015). In 2004, Takamizawa et al. (2004) reported a reduced expression of let-7 in lung cancer cells. The members of let-7 family are known as tumor suppressors that negatively regulate oncogenes such as *KRAS*, *c-MYC*, *CDK6*, *HOXA9*, *TGFBR1*, *BCL-XL,* and *MAP4K3* (Wang et al. 2012). The identification of the let-7 target, *RAS*, was the first evidence that miRNAs can negatively regulate the expression of oncogenes. Johnson et al. (2005) reported that overexpression of let-7 directly represses *RAS* protein levels. *RAS* family are proto-oncogenes including the three human *RAS* genes *HRAS*, *KRAS* and *NRAS*. The *RAS* family members are associated with numerous signaling pathways, including Ras/PI3K/Akt and Ras/Raf/MEK/MAPK. *RAS* oncogenes encode G-proteins that a play critical role in controlling cellular signal transduction pathways. They cooperate in the regulation of cell proliferation, growth, differentiation, migration, and survival (Downward 2003). *KRAS* mutations are present in about 30 % of NSCLCs. Conversely, *HRAS* and *NRAS* mutations occur much less frequently in lung cancer (Thiagalingam 2015). A reduced let-7 expression leads to overexpression of *RAS* and it is significantly associated with shortened post-operative survival, independent of disease stage (Takamizawa et al. 2004).

Overexpression of miR-21 is frequent in NSCLC and it enhances tumorigenesis through inhibition of negative regulators of the Ras/MEK/ERK pathway and inhibition of apoptosis. Expression of miR-21 increases with activation of the oncogenic *KRAS* (Hatley et al. 2010). Zhang et al. (2010a) showed that miR-21 represses *PTEN* (phosphatase and tensin homolog) and stimulates growth and invasion in NSCLC cell lines. *PTEN* is a tumor suppressor that inhibits cell invasion by blocking the expression of several matrix metalloproteases (Meng et al. 2007). Seike et al. (2009) reported that miR-21 is upregulated under conditions, in which EGFR signaling pathway is activated, and it is suggested to be related to lung cancer development in never-smokers. A recent study of Yang et al. (2015) shows that inhibition of miR-21 expression reduces proliferation, migration, and invasion of adenocarcinomic human alveolar basal epithelial cells by upregulating the expression of programmed cell death 4 (PDCD4). The PDCD4 is known as a tumor

suppressor that inhibits invasion of cells, promotes cell apoptosis, reduces neoplastic transformation, and tumorigenesis (Lankat-Buttgereit and Göke 2009). The miR-21 can be useful as a potential diagnostic and prognostic indicator for NSCLC (Zhao et al. 2015).

2.3 PI3K/AKT Pathway

The PI3K/Akt pathway is an important transduction pathway in NSCLCs and SCLCs. This signaling pathway is involved in the regulation of cell proliferation, survival, differentiation, and motility. It is activated through a variety membrane receptors including EGFR, HER2, IGF-1R, and VEGF (Cooper et al. 2013). The activity of PI3K/Akt is repressed by the tumor suppressive miR-126 by targeted binding to the 3′-untraslated region of *PI3KR2* mRNA. Overexpression of miR-126 in NSCLC cell lines inhibits cell proliferation and tumor growth. Patients with low expression of miR-126 have a significantly poorer survival time than those with high miR-126 expression (Yang et al. 2012). Overexpression of miR-126 in lung cancer also reduces the CRK protein that plays a role in decreasing adhesion, migration, and invasion (Crawford et al. 2008). The CRK protein mediates several intracellular signaling pathways in the processes of cell growth, motility, differentiation, and adhesion. A recent study of Yang et al. (2015) shows that miR-126 can inhibit the proliferation of adenocarcinomic human alveolar basal epithelial cells *via* regulation of the VEGF protein level. These authors demonstrated that *VEGF* is a target gene for the miR-126.

2.4 *MYC* Oncogene

A frequently activated proto-oncogene in tumors is *MYC* (Albihn et al. 2010). The *MYC* proto-oncogene is one of the major downstream effectors of the RAS/RAF/MEK/MAPK pathway. The MYC proteins are involved in the regulation of cell cycle, normal cell growth, and apoptosis. Activated *MYC* oncogene increases the synthesis of his target proteins and by doing so it increases cell growth, division, and survival of cells (Ruggero 2009). The *C-MYC* oncogene is targeted by miR-145 that dramatically suppresses the *C-MYC*/*eIF4E* pathway. Overexpression of miR-145 inhibites the cell growth and blocks the G1/S transition in the cell cycle (Chen et al. 2010).

Other investigators have found a connection between the miR-17-92 cluster and the *C-MYC* oncogene which is frequently increased in SCLC (O'Donnell et al. 2005). The miR-17-92 cluster is comprised of six miRNAs: miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a-1 (Qi and Mu 2012). This cluster directly targets the hypoxia-inducible factor-1α. Overexpression of *C-MYC* leads to downregulation of HIF-1α and induction of miR-17-92. This suggests that induction of miR-17-92 may play a part in the *C-MYC*-mediated repression of HIF-1α (Taguchi et al. 2008). In addition, Ebi et al. (2009) reported the association of miR-17-92 overexpression with inactivation of the retinoblastoma protein (RB). These results suggest that the miRNA cluster may be a potential therapeutic target in lung cancer.

2.5 *TP 53*

The tumor suppressor gene *TP53* acts as a transcription factor controlling the expression of numerous different genes. *TP53* has a critical role in the regulation of the cell cycle and induction of apoptosis (Guz et al. 2014). *TP53* inactivation is one of the most significant genetic abnormalities in lung cancer. It occurs in 90 % of SCLC and about 65 % of NSCLC (Cooper et al. 2013; Wistuba et al. 2000). Members of the miR-34 family reportedly target the *TP53* transcription directly. Overexpression of miR-34 induces apoptosis and cell cycle arrest (He et al. 2007). miR-34 family members are involved in the cell cycle control, apoptosis, and senescence of the cells also through a specific targeting of *BCL-2*, *MYC,* and *MET* genes (Bommer et al. 2007). Mudduluru et al. (2011) reported that miR-34a inversely correlates with

the receptor tyrosin kinase AXL protein in NSCLC cell lines, which induces proliferation, migration, and invasion of cancer.

Zhang et al. (2010b) reported that miR-221 and miR-222 directly target PUMA (p53 up-regulated modulator of apoptosis) expression. A reduction of miR-221 and miR-222 inhibits cell proliferation and induces mitochondrial-mediated apoptosis. Yamashita et al. (2015) unraveled the tumor suppressive effects of miR-221 and miR-222 in lung cancer cells through intra-S-phase arrest and/or apoptosis of the cells.

3 Conclusions

MicroRNAs play an important role in cancer development by controlling cell differentiation, proliferation, apoptosis, invasion, and metastasis. The understanding of the molecular mechanisms of microRNA-regulated pathogenesis in cancer and microRNAs regulation of the multiple stages of cancerogenesis, i.e., initiation, promotion, malignant conversion, progression and, metastasis, is still unclear. One possible mechanism is that microRNAs regulate cancer development by targeting tumor suppressors and oncogenes. In this review, we have discussed how dysregulated expression of microRNAs could contribute to the maintenance of lung cancer through down-regulation of tumor suppressors and up-regulation of oncogenes. MicroRNAs can be used as novel biomarkers for the diagnosis, prognosis, and prediction of the response to treatment; the role that gets unraveled in studies on microRNA expression profiles in cancer cells and tissues. The microRNA expression profiling has the potential to differentiate between normal and cancer cells and between different cancer subtypes.

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Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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Laryngeal Cancer: 12-Year Experience of a Single Center

V. Calkovsky, P. Wallenfels, A. Calkovska, and A. Hajtman

Abstract

Laryngeal cancer is about the twentieth most common cancer in the world and more than 150,000 new cases are diagnosed annually. The aim of the study was to evaluate the history, diagnostics, treatment outcomes, and prognosis in patients with laryngeal cancer in Northern Slovakia. We analyzed retrospectively 227 patients (207 males, 20 females) with laryngeal carcinoma treated in the period 2003–2014 at the Clinic of Otorhinolaryngology and Head and Neck Surgery of the Jessenius Faculty of Medicine and Martin University Hospital in Martin, Slovakia. The majority of patients were in the sixth (38.0 %) and seventh decade of life (30.8 %). Two hundred and seventeen patients (95.6 %) were smokers or ex-smokers. Sixty-six percent of patients were diagnosed with glottic or transglottic carcinoma, related probably to the anatomical structure of the larynx and exposure to inhalation pollutants. It is alarming that the majority of patients with malignant laryngeal disease were admitted to the hospital in advanced stages. In 151 (66.5 %) of patients, the extent of infiltration was T3 or T4, and 156 (68 %) patients were in disease stage III and IV. The incidence and mortality of laryngeal cancer suggest the need to intensify the prevention and to search for an early clinical stage of laryngeal cancer using a targeted screening.

Keywords

Carcinoma • Complications • Diagnostics • Laryngectomy • Larynx • Prognosis • Risk factors

V. Calkovsky (✉), P. Wallenfels, and A. Hajtman
Clinic of Otorhinolaryngology and Head and Neck Surgery, Jessenius Faculty of Medicine, Comenius University and Martin University Hospital,
2 Kollarova St., 036 01 Martin, Slovakia
e-mail: calkovsky@jfmed.uniba.sk

A. Calkovska
Department of Physiology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

Biomedical Center Martin, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

1 Introduction

Laryngeal cancer is about the twentieth most common cancer in the world and more than 150,000 new cases are diagnosed annually (WCRFI 2012). The overall incidence of laryngeal cancer in the EU is 4.4/100,000 and mortality is 1.8/100,000 according to the WHO data of 2012 (ECO 2012). The incidence and mortality are lower in women compared with men in most European countries (Bosetti et al. 2006). Laryngeal cancer is a multifactorial disease that is linked to various factors related to life-style. Tobacco smoking and alcohol drinking are two major risk factors for laryngeal cancer in the developed countries (Bray et al. 2000), but other risk factors, e.g., asbestos, have been implicated (Peng et al. 2015). The association between gene polymorphism and susceptibility to laryngeal cancer has also been reported (Yu et al. 2015).

Controversies exist concerning the management of laryngeal cancer. The treatment has to be multidisciplinary and requires different approaches. Partial laryngectomy, laser resection, or radiation therapy are preferentially used in early laryngeal tumors, while subtotal or total laryngectomy, followed by radiotherapy, is usually applied in the advanced disease (Forastiere et al. 2015). The prevention of voice disorders and dysphagia, which are the most frequent complications in patients undergoing surgical treatment, improve the patient's long-term quality of life (Calkovsky and Hajtman 2015). To improve the outcome and prognosis of laryngeal cancer, extensive research has been done in this field (Li and Wang 2014; Meijer et al. 2014).

The aim of the present article was to gain insight into different medical statuses of patients hospitalized in University Hospital Martin in Slovakia with laryngeal cancer. We addressed the issue by evaluating the patient's history, diagnostic procedures, the extent and success of surgical treatment, and the possible complications and their resolution.

2 Methods

The study was conducted in comport with the Ethical Standards of the Helsinki Declaration and was approved by the Institutional Ethics Committee. Two hundred and twenty-seven patients (207 males, 20 females) with malignant laryngeal carcinoma, who underwent surgery in the period of 2003–2014 at the Clinic of Otorhinolaryngology and Head and Neck Surgery of the Jessenius Faculty of Medicine, Comenius University and Martin University Hospital in Martin, Slovakia, were retrospectively evaluated. Data on the patient's history, risk factors, treatment, and the outcomes were acquired from medical records.

The inclusion criterion was newly diagnosed and histologically verified laryngeal cancer. We evaluated the occurrence of laryngeal tumors in males and females, the age distribution of hospitalized patients at the time of diagnosis, the treatment outcome, and the proportion of smokers. Clinical staging of cancer was determined according to the tumor-node-metastasis (TNM) classification system in compliance with the European Laryngological Society recommendations (Simo et al. 2014). The type and efficiency of surgical treatment, 5-year follow-up in the years of 2003–2007, and 3-year survival rate in 2008–2010 were also evaluated.

3 Results

3.1 Demographics and Smoking

The patient age range was 23–81 years, with the mean of 61.8 ± 8.4 years. The ratio between males and females was 9:1. All patients were diagnosed with laryngeal squamous cell carcinoma (LSCC) and no other histological types of tumors were identified. The distribution of patients by age is shown in Table 1. There were 186 smokers (82.0 %), 31 (13.6 %) ex-smokers, and 10 (4.4 %) never smokers. In the subgroup of ex-smokers, the longest period of no smoking amounted to 32 years.

3.2 Tumor Location, Staging, and Grading

The majority of patients had the transglottic location of a tumor. In a 12-year period we identified just one case of the subglottic location of a tumor (Table 2).

According to the disease extent, the majority of patients (42.0 %) had tumor in stage T3. The other stages were present as follows: T4 in 26.0 %, T2 in 21.0 %, and T1 in 11.0 % of patients. The regional lymph nodes were unaffected (N0) in the majority of patients (65.0 %). A distinct infliction of lymph nodes was present in just two patients. Metastases were present in 26.0 % of cases of the advanced stages N2-N3 and in 9 % of stage N1 patients. The degree of tumor cell differentiation (grading) was as follows: G1 in 14.5 %, G2 in 68.0 %, and G3 in 17.5 % of patients. TNM classification was comprehensively evaluated and the stage of laryngeal cancer was determined in every patient. The distribution of disease stages is shown in Fig. 1.

Table 1 Distribution of patients by age

Decades of life	n	%
21–30 years	1	0.4
31–40 years	1	0.4
41–50 years	34	15.0
51–60 years	86	38.0
61–70 years	70	30.8
71–80 years	32	14.1
81–90 years	3	1.3
All	227	100

n number of patients

Table 2 Distribution of patients by tumor location

	n	%
Glottic	41	18.1
Supraglottic	28	12.3
Subglottic	1	0.4
Transglottic	111	48.9
Lesion exceeding larynx margins	46	20.3
All	227	100

n number of patients

3.3 Surgical Treatment

One hundred forty one patients were treated surgically. Twenty three patients declined the surgery. Sixty three patients were considered inoperable due to the disease extent or comorbidities (Table 3).

3.4 Survival Rate

The 5-year 2003–2007 survival was evaluated in 77 patients. Eleven patients were excluded from the evaluation due to incomplete records, so that 66 patients were included in the final analysis. A 5-year survival in relation to the disease stage is

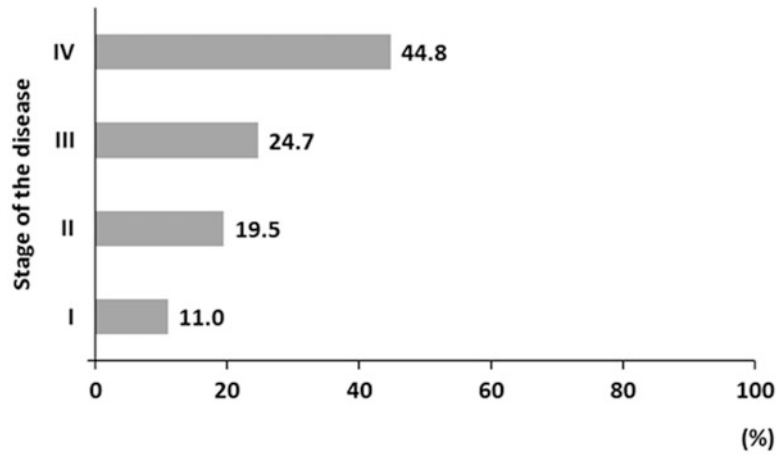

Fig. 1 Stages of laryngeal tumors