REVIEW ARTICLE

Molecular Characterisation of Sinonasal Carcinomas and Their Clinical Implications

Fernando López, José Luis Llorente, María Costales, Cristina García-Inclán, Jhudit Pérez-Escuredo, César Álvarez-Marcos, Mario Hermsen, Carlos Suárez

Servicio de Otorrinolaringología, Instituto Universitario de Oncología del Principado de Asturias, Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain

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Abstract Sinonasal carcinomas are rare tumours with an unfavourable prognosis whose management is difficult and complex, leading to high morbidity and mortality despite improvements in the field of surgery and radiotherapy. An elevated number of these tumours can be attributed to occupational exposure. In comparison with other head and neck malignancies, studies of molecular changes in these tumours are infrequent. This review was focused on findings about the epidemiology and molecular and phenotypic characterisation of sinonasal carcinomas, which can potentially be useful for diagnosis and treatment. The increasing knowledge about the molecular biology that underlies their carcinogenesis may help to identify precursor lesions, prognostic markers and markers that predict chemoradiotherapy response and, finally, to identify potential molecular targets that will expand treatment options.

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Caracterización molecular de los carcinomas nasosinuasales y sus implicaciones clínicas

Resumen Los carcinomas nasosinuasales son tumores infrecuentes con mal pronóstico y cuyo manejo es difícil y complejo, conllevando una elevada morbimortalidad a pesar de tratamientos quirúrgicos agresivos y administración de radio y quimioterapia. Son tumores frecuentemente relacionados con exposición profesional a carcionógenos. A diferencia de otros tumores de cabeza y cuello los estudios acerca de los cambios genético-moleculares de estos tumores son escasos. Esta revisión se centra en los hallazgos acerca de la epidemiología y la caracterización molecular y fenotípica de los carcinomas nasosinuasales, y sus posibles implicaciones en el diagnóstico y tratamiento de los mismos. El progresivo conocimiento acerca de la biología molecular que subyace a su oncogénesis ayudaría a identificar lesiones precursoras, marcadores pronósticos y
Introduction

Sinonasal carcinomas (SNC) are infrequent neoplasms, constituting less than 3% of the tumours arising from the head and neck area. Their epidemiology, histopathology and clinical characteristics are different from the rest of the malignant neoplasms of the head and neck. Diagnosis and treatment of these tumours pose several problems due to their very low incidence, histological diversity, production of non-specific symptoms in the early stages that can simulate an inflammatory process and, because they have a variable prognosis depending on their histology, location and staging. Their central facial location and the existence of adjacent structures such as the orbits, oral cavity and skull base make their treatment difficult and complex, involving high morbidity and mortality.

The SNC are a group of aggressive tumours. However, progressively growing knowledge as to the genetic-molecular mechanisms that underlie their oncogenesis enables us to identify tumour subgroups with a specific biological behaviour. This is important in trying to establish diagnostic strategies and individualised therapies.

This article presents a review of the current state of knowledge on the molecular characterisation of the SNC, especially the sinonasal squamous cell carcinoma (SNSCC) and sinonasal adenocarcinoma (SNA), and their possible implication in relation with diagnosis and therapeutic possibilities.

Incidence and Survival

The incidence rate is approximately 1 case per 100,000 inhabitants and year (0.7 for males and 0.3 for females). This incidence has remained constant in the last few years, although it has dropped slightly for males. The mean age of appearance is between the 60s and the 70s.

The tumours of epithelial origin such as SNSCC (50%), SNA (13%) and undifferentiated carcinomas (3%) are the most frequent subtypes. Nevertheless, in our personal series SNA occur more frequently than SNSCC. The nostril is the most frequent location (45%), followed by the maxillary sinus (36%) and the ethmoid (10%). However, given the proximity of these anatomical areas and the fact that tumours frequently present in advanced stages, it is not easy to identify the exact spot from which they started. In addition, a significant number of tumours affect more than 1 adjacent location at the moment of diagnosis (Table 1).

Overall mean 5-year survival for SNC ranges between 20% and 70%, depending on histology stage and location (Table 1). Survival has increased in the last few decades, although not significantly. The SNA are the only tumours than have truly improved the survival rate (currently 60% at 5 years).

Aetiopathogenic Factors

The aetiopathogenic mechanisms are not known with precision, even though there are different environmental, viral and physical carcinogens that might be involved. Although in a less evident manner than with laryngopharyngeal carcinoma, there is sufficient evidence that tobacco smoke produces SNC.

In up to 40% of SNC, a relationship has been found to exposure to different occupational factors in the European male population. The overall risk attributable to developing SNC associated with various occupations is 33%. For the SNA, the risk is 77% and, for the SNSCC, it is 22%.

Exposure to Wood Dust

Workers in the wood industry have been seen to have 3 times greater risk of developing SNC. A few histological subtypes, such as SNA, are related to prolonged exposure to dust from hard woods (oak and beech) and SNSCC is related to exposure to soft woods (fir, pine). However, Demers et al. observed that the risk was greater for SNA and not for SNSCC. Cantu et al. found history of wood dust exposure in 97% of the cases of SNA. However, only 20% of SNA cases in Non-European patients have a history of exposure to wood. This fact could be explained by phenomena of genetic susceptibility (still underdemonstrated) or of different types of wood, additives or wood origins.

Other Professional Exposure

Various studies have estimated that workers in the footwear industry have 10 times higher risk of having SNA than the general population. Other chemical substances that have been associated with developing SNC, preferably SNSCC, are formaldehyde, chrome, nickel, radium and various substances used in the textile industry.

Biological Factors

The presence of human papillomavirus (HPV) 16 and 18 has been demonstrated in 20% of SNC. It seems that HPV-positive tumours have a higher proliferation rate and are positive for staining with p16, while the HPV-negative ones are p16-negative and have elevated p53 expression. Similarly to what is described in the oropharynx, HPV-positive tumours form a specific histological and molecular subgroup, given that there is better prognosis and they respond better to chemoradiotherapy. However, in SNA, HPV has not been seen to be involved in the oncogenesis. Pérez Escuredo et al. have studied the presence of HPV, Epstein–Barr virus (EBV), herpes simplex virus (HSV), varicella zoster...
virus (VZV), adenovirus and of cytomegalovirus (CMV) in intestinal-type adenocarcinoma (ITAC). The presence of DNA from HSV, HPV, HSV, adenovirus and VZV was lacking and only 8% and 2% of the cases were carriers of EBV and CMV respectively. Given these results, the virus studies do not appear to play any role at all in SNA aetiology.

**Histopathology**

The SNC tumours present great histological variability. This diversity sometimes makes it impossible to assess homogenous patient groups. In fact, this is a frequent occurrence in some SNC series published.

**Adenocarcinoma**

These are divided into 2 large groups: intestinal-type SNA (ITAC), which comprise the most frequent subtype, and non-intestinal-type SNA. Differentiation is possible using immunohistochemical study of epithelial differentiation markers. These markers are characteristically expressed in ITAC cases, but not in non-intestinal-type SNA. In turn, ITAC are divided into various histological subtypes (papillary, colonic, solid, mucinous and mixed). This is especially relevant in prognosis, because the first 2 subtypes have a more indolent clinical behaviour.

No precursor histological lesions have been identified for these tumours. Vivanco et al. analysed the presence of histological changes in healthy mucosa adjacent to the tumour in patients with ITAC. They observed dysplastic changes in 14% of the cases in the study. Although it could be thought that tumour growth would eliminate the surrounding dysplastic tissue, the researchers found no differences in the coexistence of dysplasia between tumours in early and advanced stages. Some authors have indicated that the presence of cuboid metaplasia could be a precursor lesion of ITAC. Vivanco et al. found this presence in 57% of the cases and, in those in which it coexisted with dysplasia, the tumour recurrence rate was greater. Likewise, in 8% of the cases they observed intestinal metaplasia, which would reflect its relationship with colorectal adenocarcinomas. Kennedy et al. suggested that respiratory epithelium, either through cuboidal or intestinal metaplasia, would suffer dysplastic changes before being transformed into ITAC. Other authors suggested that the goblet cell hyperplasia observed in the mucosa of workers in textile industries could be a precursor lesion. Consequently, although further studies are needed, it seems that there are probably precursor lesions for ITAC.

**Epidermoid Carcinoma**

Although it has not been confirmed, SNSCC might arise from a metaplastic transformation of normal respiratory epithelium. Many studies have attempted to demonstrate that sinonasal inverted papilloma (IP) could be considered as precursor lesions, given that there are synchronous and metachronous SNSCC in 7% and 4% of the cases respectively. However, Califano et al. observed that, although IP are monoclonal lesions, they do not show the characteristic phenotypic profile of a precursor lesion; nor do they carry the genetic alterations associated with malignant transformation observed in the rest of the aerodigestive tract.

**Undifferentiated Carcinoma**

Specific SNC can have undifferentiated or only slightly differentiated histology. Although differential diagnosis can be complicated, correctly classifying this group of tumours is essential because treatment and prognosis can vary. A clear example is the different biological behaviour between esthesioneuroblastoma (ENB) and undifferentiated carcinoma. On occasions, a single tumour can share features of more than 1 entity. Genetic and immunohistochemical...
analysis make precise diagnosis of these neoplasms easier (Table 2).  

Genetic Tumour Profile Analysis

Intestinal-Type Adenocarcinoma

Initially, due to their histopathological similarity, most of the studies centred on a limited number of genes and proteins implicated in the oncogenesis of colorectal adenocarcinoma and, in certain cases, with contradictory results.  Some genes frequently altered in colorectal adenocarcinoma are TP53, KRAS, APC and β-catenin; their analysis showed that the incidence of alterations in the ITAC was lower than expected.

After that, to study the ITAC genome overall, work using comparative genomic hybridisation (CGH) was performed; these studies showed a different genetic pattern from that observed in other head and neck tumours, but it was similar to that of colorectal adenocarcinoma.  In addition, it was observed that the tumours with the greatest frequency of gains presented a higher histological grade, which would indicate that genetic amplification was involved in ITAC differentiation.

In 2009 Hermansen et al. using CGH microarrays, detected that the most frequent gains were located 5p15, 20q13 and 8q24, while the losses were in 4q31-qter, 18q12-22, 8p12-pTER and 5q11-qter. This pattern had certain similarities with colorectal adenocarcinoma. However, the gains in chromosomes 3, 5 and 11q13 observed in ITAC are infrequent in colorectal tumours. Microarray findings are more precise and make it possible to locate genetic alterations more exactly, but they basically corroborated the CGH findings. For example, the gains in 8q observed using CGH were detailed by microarrays to the 8q24.3 region where gene PTP4A3 is located, but not MYC or FAK (which are in 8q24). In contrast to what happens in 10% of colorectal adenocarcinoma, instability of microsatellites was not confirmed. In addition, in the cases in which few genetic changes were found, the DNA content was diploid and had more beneficial clinical-pathological characteristics (papillary or colonic histology, absence of intracranial invasion, less metastasis and greater overall survival). Consequently, molecular analysis would seem to make it possible to identify a subgroup of patients with better prognosis. The profile of chromosome gains and losses revealed data in agreement with what had been seen previously using Multiplex ligation-dependent probe amplification (MLPA) at the genetic level.

Squamous Cell Carcinoma

Due to its low incidence, hardly any studies have been published on the molecular changes underlying the oncogenesis of this type, in contrast to the multitude of publications related to other squamous cell carcinomas (SCC) of the head and neck. López et al. published a study that showed that the genetic profile of SNSSC shared similarities with laryngopharyngeal SCC, such as the amplifications in regions 11q13 (CCND1, CTNN1), 7p12 (EGFR) and 11p13 (CD44), despite not having as clear an epidemiological
relationship with tobacco and alcohol use. This finding could be relevant when making clinical decisions and when using biological therapies, given that the advances in the more frequent tumours such as those of the larynx could be used in SNSCC cases.

In contrast to what was observed with SNA, microsatellite instability could indeed play an important role in the oncogenesis of SNSCC.

Genetic Analysis

**TP53**

Alterations in TP53 constitute a frequent event in the initial stages in tumours of head and neck. In the SNC the frequency of mutations range between 18% and 77% and vary with histology, being higher in SNA than in SNSCC. Holmila et al. described a specific pattern of TP53 mutations in ITAC, which might be related to its relationship with exposure to wood dust. The TP53 alterations could be used as a screening method in patients exposed to wood dust. In both patients with SNSCC and ITAC, the predictive role of TP53 mutations in response to chemotherapy was studied, observing that these mutations increased in chemoresistance.

**EGFR and HER2**

Various studies have shown that EGFR and HER2 play an important role in the pathogenesis of squamous cell carcinomas of the head and neck, amplification and/or overexpression being associated with poor prognosis and greater relapse rates. The existence of biological therapies aimed at both receptors give their study special relevance.

There are few studies that show variable expression of both proteins in SNSCC. In addition, there is discrepancy as to their validity as prognostic factors in SNSCC. López et al. detected a gain in the number of copies of EGFR in 44% of the cases and protein overexpression in 39%, while 21% showed HER2 gains and 7% overexpression of the protein. Overexpression of EGFR is significantly related to the presence of ganglion metastasis and orbit involvement, while HER2 overexpression is related to intracranial invasion. These data, if they are confirmed in other studies, could be used to make therapeutic decisions. In addition, the data would indicate that a subgroup of patients with SNSCC could benefit from therapies aimed against receptors.

Franchi et al. studied the state of EGFR in ITAC. They observed EGFR amplification and overexpression in 33% of the cases. These data are similar to those obtained in a study performed in our laboratory in which we found EGFR overexpression in 21% of the cases and absence of mutations in the gene. We found a lack of associations with prognostic-clinical factors as well. Gallo et al. analysed HER2 expression in ITAC, finding overexpression in a third of the cases, which had a more aggressive clinical course. These preliminary data indicate that the activation of both EGFR as well as HER2 might consequently be involved in the oncogenesis of ITAC and that therapies aimed against both receptors could be researched prospectively in this subgroup of patients.

**KRAS and BRAF**

Los proto-oncogenes of the RAS family are active in approximately 20% of human tumours, through specific mutations. Knowing the state of KRAS and BRAF is essential, given that their activation is one of the phenomenon that produce resistance to therapies aimed against EGFR. The frequency of KRAS and BRAF mutations in SNC is minimal, which suggests that both genes play a limited role in the oncogenesis of these tumours.

Protein Analysis

Altered expression of annexins has been implicated in tumour development and progression. Rodrigo et al. studied their expression in ITAC, observing the loss of annexin A1 expression in 91% of the cases in comparison with normal epithelium. Annexin A2 expression was more heterogeneous and a loss of expression was only seen in 33% of the tumours, being associated with more aggressive histopathological subtypes and a drop in survival. However, the impact on survival might be an indirect consequence of association with the histological type.

Proteins related to controlling the cell cycle and the phenomenon of apoptosis and angiogenesis have also been studied. Bandoh et al. observed that SNSCC cases with loss of p21 expression, due to mutations in p53, had a higher proliferation index, lower survival and worse response to chemotherapy. These same authors found that a low apoptotic index was an independent factor for poor prognosis and that, in tumours with a high level of spontaneous apoptosis induced by Bax overexpression, there was greater sensitivity to chemoradiotherapy, which conditions a factor for favourable prognosis; while those with TP53 mutation were more chemoradiotherapy-resistant, so they had worse prognosis. In the same group of tumours, it was observed that increasing the expression of the receptor for vascular endothelium growth factor (VEGF) was related to an increase in vascular density, a decrease in apoptosis and the development of metastasis; in contrast, the expression of fibroblast growth factor (FGF) was related to resistance to chemoradiotherapy. Both data, consequently were considered indicators of poor prognosis.

Signal Pathway Analysis

The canonical Wnt pathway is one of the most important pathogenic pathways for the development of colorectal adenocarcinomas. Díaz et al. detected that this route is activated in approximately a third of the patients with ITAC, while it is activated in almost 80% in colorectal adenocarcinoma. One of the key proteins in this pathway is β-catenin, whose nuclear expression has been shown to be an independent factor for poor prognosis.
Table 3  Key Aspects.

1. Sinonasal carcinomas are infrequent tumours, of unfavourable prognosis, in spite of the advances in surgical techniques and radiotherapy
2. A precise anatomopathological diagnosis will help to select the most appropriate treatment. Applying studies in immunohistochemistry and molecular diagnosis is essential to achieve such a diagnosis
3. Up to the present, no histological lesion or molecular alteration that constitutes a precursor of these tumours has been identified
4. Studying the genetic profile of squamous cell carcinoma and sinonasal adenocarcinoma makes it possible to identify a subgroup of tumours susceptible to treatment with biological therapies
5. Obtaining cell lines and orthotopic murine models will help to understand tumour biology of sinonasal carcinoma
6. To increase knowledge on the molecular mechanisms that participate in the carcinogenesis of these tumours, carrying out studies with a greater number of cases is advisable, which will make it possible to extract consistent conclusions for clinical application

Cell Lines and Animal Models

Stable cell lines, with a few limitations, make it possible for us to maintain and study in vitro the biological characteristics of the primary tumour. Until we established and characterised a cell line from ITAC and 6 lines from SNSSC (pending publication) in our laboratory, there were no descriptions in the literature of any cell lines derived from SNA (because they are cells that are difficult to cultivate) or from SNSSC (because the published cell lines were from tumours in the maxillary infrastructure). The established cell lines have a high proliferation rate and elevated invasive potential, and their genomic profiles coincide with the original primary tumour from which they were derived. Together with these gene and protein data, these cell lines constitute a useful tool for preclinical assays of new therapy possibilities.

In vivo animal models constitute a further step in the dynamic study of carcinogenesis and studies on therapy efficacy. The first sinonasal orthotopic murine model in mice was published by Gelbard et al. However, they used cell lines from floor-of-mouth squamous cell carcinoma and from cystic adenoid carcinoma. There is currently no orthotopic animal model for SNA. A model of these characteristics becomes more relevant due to the continuous studies on prevention and provocation of these tumours by exposure to wood dust and the logical occupational consequences that it would bring.

Perspectives for the Future

Bearing in mind the limitations derived from their scant incidence, molecular-genetic characterisation of SNA and SNSSC has been carried out progressively. Nevertheless, performing studies on specific markers in larger patient series is needed to confirm or rule out previous findings. The studies should be aimed at identifying morphological lesions and/or the molecular markers of the precursor lesions and early stages, markers related to prognostic factors and chemoradiotherapy response factors and, finally, at describing new therapeutic targets (Table 3).

Conflict of Interests

The authors have no conflict of interest to declare.

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