Nasosinusal Adenocarcinoma: Molecular and Genetic Analysis by MLPA

José L. Llorente,[a,b] Patricia Aldama,[a] César Álvarez-Marcos,[b] Jhudit Escudero,[b] Marta Alonso-Guervós,[b] Florentino Fresno,[c] Carlos Suárez,[a] and Mario Hermsen[b]
[a]Servicio de Otorrinolaringología, Hospital Central de Asturias, Oviedo, Asturias, Spain
[b]Instituto Oncológico del Principado de Asturias, Universidad de Oviedo, Oviedo, Asturias, Spain
[c]Servicio de Anatomía Patológica, Hospital Central de Asturias, Oviedo, Asturias, Spain

Introduction and aim: Intestinal-type sinonasal adenocarcinomas (ITACs) are rare epithelial tumours, primarily originating in the nasal cavities and paranasal sinuses, and characterized by glandular structures. The aims of this study are: to determine the genetic alterations in ITACs by MLPA (Multiplex ligation-dependent probe amplification) and to correlate the findings to the clinical behaviour and follow-up information of the patients.
Material and method: We performed a longitudinal prospective study on 20 patients with ITAC, seen in our department between 1998 and 2004. DNA was extracted from primary tumour samples and analyzed by MLPA.
Results: The T stage of our series was T2: 4 (20%), T3: 6 (30%), T4a: 3 (15%), and T4b: 7 (35%). All cases initially were N0 and M0. Seventeen patients (85%) had professional exposure to wood dust. All patients underwent surgical intervention and 70% received complementary radiotherapy. Overall 5 and 10 year survival was 42% and 22%, respectively. Gains were found most frequently for *PTP4A3* and *PDCD8* (65%), *TNRFSF7* (50%), *RECQL4* and *LMO2* (45%), and losses for *BCL2* (70%), *IL13* (55%), *ABCB1* and *RB1* (50%), *PIK3CA* and *CDH1* (45%).
Conclusions: Losses of *F3, MIF*, and *BRCA1* significantly correlated with the posterior development of metastases and with worse survival. Also gains of *PIK3CA, UTY*, and *RELA* correlated with poor clinical outcome. Losses of *BRCA1* and *F3* were significant in multivariate analysis.

Key words: Intestinal-type sinonasal adenocarcinomas. MLPA. Sinonasal adenocarcinoma.

Análisis genético molecular con MLPA en los adenocarcinomas nasosinusales

Introducción y objetivo: Los adenocarcinomas nasosinusales (ACNS) son tumores epiteliales raros, primarios de las fosas nasales y los senos paranasales, que se caracterizan por presentar estructuras glandulares. Los objetivos de este trabajo son: determinar las alteraciones génicas en los ACNS mediante MLPA *(multiplex ligation-dependent probe amplification)* y establecer la relación entre los cambios génicos del tumor y el comportamiento clínico-evolutivo de los pacientes.
Material y método: Realizamos un estudio longitudinal prospectivo a 20 pacientes con ACNS controlados en nuestro servicio entre 1998 y 2004. A las muestras tumorales se les extrajo el ADN para realizar la MLPA.
Resultados: La categoría T de los pacientes fue para T2: 4 (20%), T3: 6 (30%), T4a: 3 (15%) y T4b: 7 (35%). Todos eran inicialmente N0 y M0. El 85% había estado expuesto, en el medio laboral, al polvo de madera. El tratamiento indicado fue quirúrgico (100%) con radioterapia complementaria (70%). La supervivencia global a los 5 y 10 años fue del 42 y el 22%, respectivamente. Las ganancias génicas más frecuentes fueron: *PTP4A3* y *PDCD8* (65%), *TNRFSF7* (50%), *RECQL4* y *LMO2* (45%); las pérdidas: *BCL2* (70%), *IL13* (55%), *ABCB1* y *RB1* (50%), *PIK3CA* y *CDH1* (45%).
Conclusiones: La aparición de metástasis se correlacionó de forma significativa con pérdidas en *F3, MIF* y *BRCA1*. La peor supervivencia con pérdidas en *F3, BCRA1* y *MIF* y ganancias en *PIK3CA, UTY* y *RELA*. En el análisis multivariable alcanzaron significación estadística las pérdidas en *BRCA1* y *F3*.

Palabras clave: Adenocarcinomas nasosinusales. MLPA. Tumores nasosinusales.

INTRODUCTION

Intestinal-type sinonasal adenocarcinomas (ITACs) are malignant epithelial tumours of the nasal passages and paranasal sinuses characterised by glandular structures.[1]

In Europe, the incidence is of 0.19 cases/100 000 inhabitants/year.[1] They are more frequent in the ethmoid

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Correspondence: Dr. J.L. Llorente Pendás.
J.M. Caso, 14. 33006 Oviedo. Asturias. España.
E-mail: llorentependas@telefonica.net

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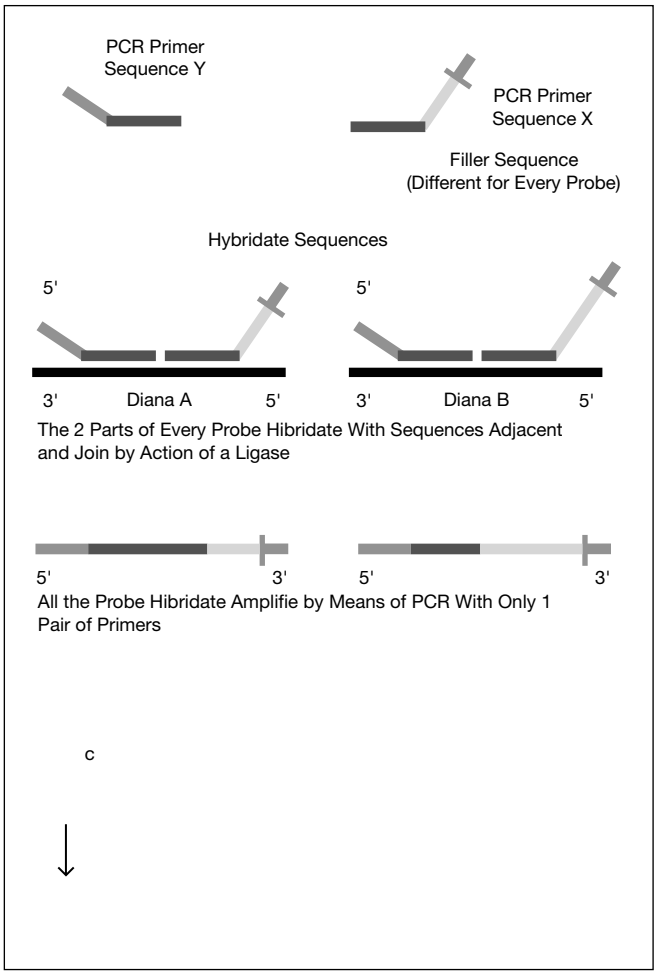

Figure 1. Molecular basis of the MLPA (top). Representation of the 41 products amplified by PCR, analyzed by capillary electrophoresis (bottom). The peak on the left represents the internal control of 94 bp (c) more to the left (↓) are the 4 internal control peaks of 64, 70, 76, and 82 bp, which confirm the high quality of the DNA from the sample and the experiment.

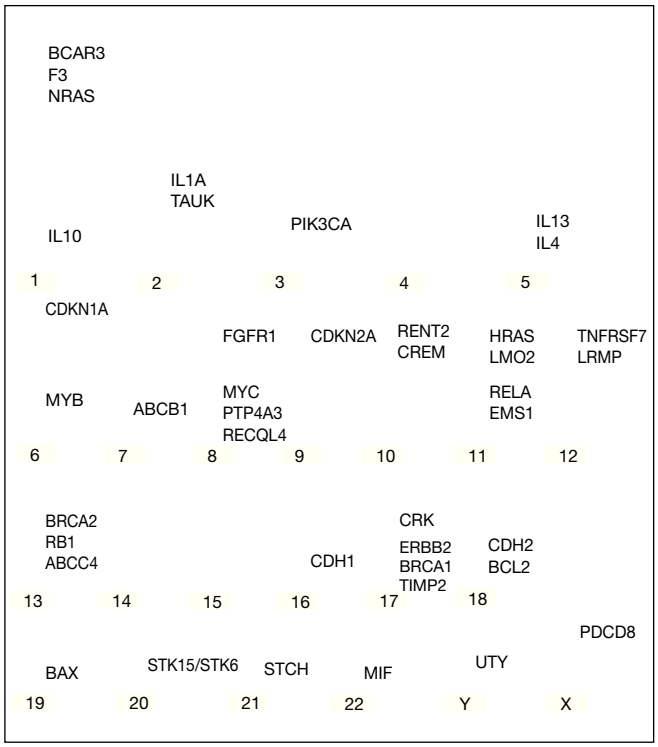

Figure 2. Chromosome distribution of the genes studied by MLPA (P005 kit).

and at the top of the nostrils (superior and middle turbinates, and middle meatus) (85%), followed by the maxillary sinus (10%), and are exceptional in the rest of the sinonasal cavities.[2,3] They appear mostly in males (4:1) aged 50-60 years.[3]

ITACs are related to occupational exposure to wood dust. It is estimated that this type of workers are 500 times more likely to develop this cancer than other males who are not exposed to it and almost 900 times more than the rest of the population.

The average exposure time to wood dust is often prolonged, from years to decades. Wood dust particles larger than 5 µm, carried by the nasal airflow are deposited in the mucosa of the target areas, while the smaller ones travel to the lower respiratory airways. The first type decrease ciliary function of the nasal cells, favouring their persistence and contact, although there is no evidence of the mutagenic effect of wood dust, which is why other oncogenic factors are presupposed such as chronic inflammation.

According to the histological classification of WHO there are 2 types of adenocarcinomas: intestinal (ITAC) and non-intestinal. Non-intestinal ones are not related to wood dust and have a different behaviour. Based on the classification of Barnes[4] and Kleinsasser et al,[5] 5 histopathological types of ITAC have been established: papillary (18%), colonic (40%), solid (20%), mucinous (alveolar, with signet cells), and mixed (transitional) (22%).

The MLPA (multiplex ligation-dependent probe amplification) is a recent molecular biology technique that amplifies and quantifies 41 different sequences of DNA or RNA in the same reaction with a single pair of primers.[6] One of its advantages is that it requires a minimal amount of nucleic acid (20 ng). The complementary oligonucleotide probes of the target sequences in the problem DNA are amplified by PCR and final products are identified by capillary or gel electrophoresis according to their different sizes (Figures 1 and 2).

The objectives of this study are to determine genetic alterations in the ITACs through MLPA and establish the relationship between genetic changes observed in the tumour and clinical behaviour and outcome of the patients.

MATERIAL AND METHOD

Patients

Data was collected from 20 clinical and histopathological ITAC patients diagnosed and treated in our department between July 1998 and March 2004. The criteria used for the selection were the following: *a)* not having received surgery,

radiotherapy, or chemotherapy previously; *b)* having the histopathology of adenocarcinoma; *c)* the sample should be collected freshly and maintained in a good condition; and *d)* the clinical monitoring should be a minimum of 6 months or until the death of the patient.

We conducted a longitudinal study designed prospectively from the collection of tumour samples during surgery, and continued with the subsequent monitoring of the patients.

The ITAC diagnosis was made by anamnesis and anterior rhinoscopic examination or endoscopy. In all cases biopsy was performed with local or general anaesthesia and imaging studies (computed tomography and magnetic resonance imaging). The final histopathological diagnosis was carried out on the piece of surgical resection by a pathologist experienced in head and neck. For the breakdown of the tumour stadium the WHO 2002, TNM system was used.

Sample Processing

The DNA was obtained from tumour tissue samples which were fresh or frozen in liquid nitrogen from the 20 patients.

The protocol used for the MLPA was the one from MRC-Holland who also supplied the reagents (http://www.mlpa.com/pages/p005pag.html) (Figure 1). We used the P005 kit with probes for genes involved in the development of tumours. Figure 2 and Table 1 describe these genes and their chromosomal location. The probes are made up of a "hybridizing" sequence of 50-70 nucleotides and another "non-hybridizing" sequence of variable length, which enables the amplified products not to match in size within a range of 130-480 bp. Each amplification product is of a unique size within that interval due to the difference in size of the filler sequence. It should be noted that the amplified products were not target the sequences of studied DNA, but the probes used for detection.

The amplified products of the 41 probes were analyzed by capillary electrophoresis (Figures 1 and 3). To this end we mixed 2 µL of amplification product with 10 µL of formamide and 0.5 µL of internal marker. The mixture is denatured by heat and subjected to capillary electrophoresis in a ABI Prism 3100 (Applied Biosystems) device. The capillaries are filled with a polymer that offers resistance to migration allowing fragments to separate by size (130-480 bp), the smaller ones will run faster than large ones. At the end of the trail there is a laser and a system to detect fluorescence signals. The final products were analyzed with the GeneScan v3.7 (Applied Biosystems) software that determines the size by interpolation with fragments of known size, quantifying each product depending on the intensity of fluorescence. The relative signal of each probe is defined by dividing the extent of the area of each peak between the sum of all areas of all the peaks of the probes from a sample. The data thus obtained should be standardized by calculating the median, standard deviation and the minimum and maximum values of all the control and tumour samples. The relation between the value of the tumour and control samples is calculated by applying the following formulae:

1. Minimum ratio = minimum value of the tumour / maximum value of control.

Table 1. Gene Gain and Loss in the 20 Patients With Sinonasal Adenocarcinomas

Gene	*Chromosomic Region*	*Loss (Cases), No. (%)*	*Gain (Cases), No. (%)*
BCAR3	1p22.1	5 (25%)	2 (10%)
F3	1p22	2 (10%)	4 (20%)
NRAS	1p13.2	0	7 (35%)
IL10	1q31	3 (15%)	0
IL1A	2q14	3 (15%)	2 (10%)
TANK	2q24	1 (5%)	4 (20%)
PIK3CA	3q26.3	9 (45%)	1 (5%)
IL13	5q31	11 (55%)	0
IL4	5q31.1	7 (35%)	0
CDKN1A	6p21.2	2 (10%)	4 (20%)
MYB	6q22	2 (10%)	6 (30%)
ABCB1	7q21	10 (50%)	1 (5%)
FGFR1	8p11.2	1 (5%)	3 (15%)
MYC	8q24.12	4 (20%)	6 (30%)
PTP4A3	8q24.3	1 (5%)	13 (65%)
RECQL4	8q24.3	0	9 (45%)
CDKN2A	9p21	8 (40%)	2 (10%)
RENT2	10p14	0	4 (20%)
CREM	10p12.1	7 (35%)	0
HRAS	11p15.5	2 (10%)	7 (35%)
LMO2	11p13	0	9 (45%)
RELA	11q13	5 (25%)	2 (10%)
EMS1	11q13	8 (40%)	2 (10%)
TNFRSF7	12p13	2 (10%)	10 (50%)
LRMP	12p12.3	4 (20%)	8 (40%)
BRCA2	13q12.3	3 (15%)	5 (25%)
RB1	13q14.3	10 (50%)	0
ABCC4	13q32	3 (15%)	4 (20%)
CDH1	16q22.1	9 (45%)	0
CRK	17p13.3	1 (5%)	3 (15%)
ERBB2	17q21.1	6 (30%)	2 (10%)
BRCA1	17q21	3 (15%)	2 (10%)
TIMP2	17q25	3 (15%)	1 (5%)
CDH2	18q11.2	0	5 (25%)
BCL2	18q21.3	14 (70%)	0
BAX	19q13.3	3 (15%)	7 (35%)
STK15/STK6	20q13.3	1 (5%)	2 (10%)
STCH	21q11	1 (5%)	7 (35%)
MIF	22q11.23	2 (10%)	4 (20%)
PDCD8	Xq25	0	13 (65%)
UTY	Yq11	6 (30%)	1 (5%)

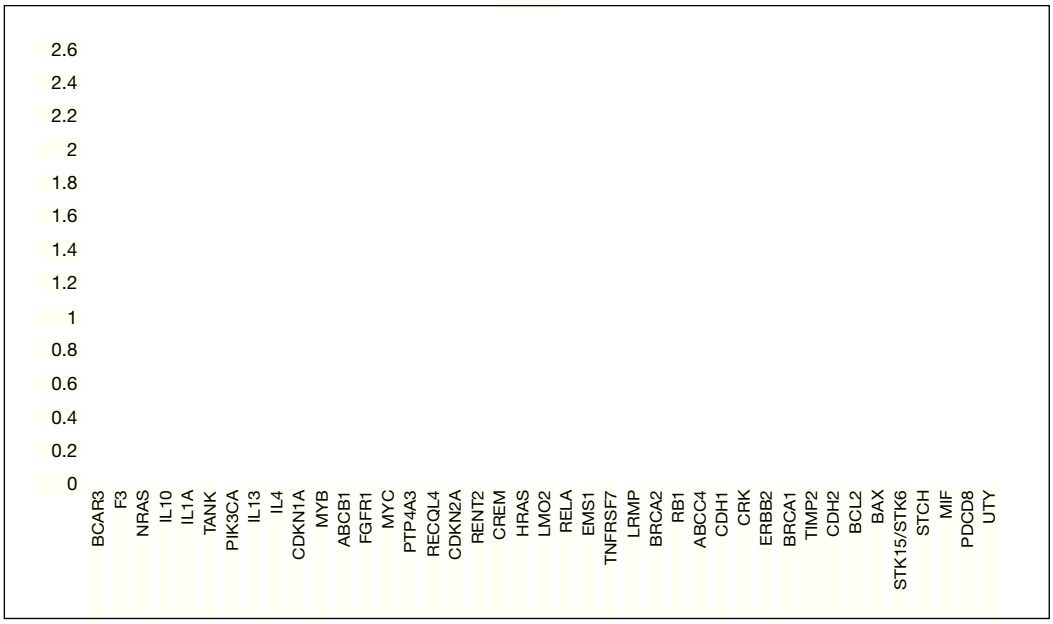

Figure 3. Bar chart of the final result obtained by MLPA for a sample of sinonasal adenocarcinomas. The grey bars represent the normalized tumour / control ratio for each probe, the black lines on each bar, the standard deviation. The loss of a probe is considered with a value <0.8 and the gain with >1.2.

2. Maximum ratio = maximum value of the tumour / minimum value of control.

3. Tumour / control ratio. It is equal to the average of the minimum ratio and the maximum ratio.

4. Standard deviation of the tumour / control ratio. It is equal to the difference between the tumour / control ratio and the minimum ratio.

The normalized analysis is presented as a bar chart of the 41 probes studied in the tumour DNA. When the tumour / control ratio is >1.2, it is interpreted as gain, and if it is <0.8, as a loss of the corresponding sequence (Figure 3).

Apart from the complementary probes to the DNA sequences that should be amplified, each MLPA kit also contains 5 control fragments called DQ (DNA Quality) (Figure 1).

Variables and Statistical Study

The clinical and pathological variables studied were: age (years), exposure time (years), tumour differentiation (good and slight), T stadium (1, 2, 3, 4a, and 4b), treatment (surgery: type, complications, radiotherapy), survival (months), recurrences, and metastases.

The gene variables studied by MLPA were the loss or gain of a particular gene in the 41 probes (kit P005) (Figure 2 and Table 1). The tumour / control ratio is interpreted as a gain if >1.2 and as loss if <0.8.

The variables described were statistically analyzed by SPSS 12.0 software for Windows (SPSS® Inc. Illinois, USA).

The association of qualitative characters was conducted with the χ^2 Pearson correlation test or Fisher exact test. The differences were calculated using the *t* test for independent samples and the McNemar test for changes and Yates correction for the Pearson χ^2 with a significance level of $P \leq .05$.

To estimate the survival the Kaplan-Meier curves were used, comparing distributions of survival through the logarithmic range test (log-rank test). A *P* value less than .05 was considered significant.

RESULTS

Clinical

The 20 patients studied were male, with an average age of 62 (range, 52-81) years. The precedent of wood dust was collected in 19 (95%); 17 (85%) had prolonged exposure, since they were carpenters or wood craftsmen; and the other 2 (10%), an occasional exposure due to being miners.

The average exposure time to wood dust was 29 (range, 5-60) years. The survival did not differ significantly depending on the exposure time being more or less than 20 years (P=.3137).

Twenty-five per cent were only smokers, 5% only drinkers, and 30% combined the 2 habits. The remaining 40% did not relate toxic habits.

All cases had the histopathological diagnosis of ITAC; 95% was of colonic type and the remaining 5%, papillary. In 50% the tumours were well differentiated; 40% moderately differentiated, and 10% slightly differentiated, without observing; with respect to survival, significant differences between well and moderately or poorly differentiated (P=.2239).

Fifty-five per cent of tumours were located in the right nostril, 40% in the left, and 5% were bilateral, always affecting the ethmoid, 60% the lamina cribosa, and 25% with intracranial extension.

The T category in the TNM staging was for T2: 4 (20%), T3: 6 (30%), T4a: 3 (15%), and T4b: 7 (35%). There were no patients with T1, N0, and M0 at the time of diagnosis. When

grouping the T2 and T3 (n=10) and T4a and T4b (n=10) survival was significantly lower in the advanced stages (*P*=.0045).

The treatment was surgical in all cases, with complementary radiotherapy in 70%. The different surgical approaches are reflected in Table 2. The survival showed no significant differences with the administration of post-operative radiotherapy, although the cases are few (*P*=.3431).

Postoperative complications were found in only 5 (25%) patients, and they were: abscess/seroma of the wound, meningitis and pneumoencephalus, pneumonia, and intracranial haemorrhage with death. The post-surgery mortality was of 5%. The 15 (75%) remaining patients showed no complications.

The number of surgical interventions for the control of the primary tumour was: 1 in 45%, 2 in 35%, and 3 in the remaining 20%. There was no significant relation observed between the number of interventions and survival (*P*=.6283).

The follow-up time was 30 months on average with an interval of 1-163 months. Thirty per cent of patients did not show tumour progression, but the remaining 70% had loco-regional recurrence (50%), metastases (temporal bone and brain) (15%), and simultaneous recurrence and metastases (5%).

It was observed that patients with metastases and recurrence had a significantly worse survival than those who did not present such an evolution (*P*=.0001).

At the end of the study, overall survival at 5 and 10 years was 42% and 22% respectively (Figure 4).

Table 2. Surgical Approaches and Size of the Primary Tumour

Type of Approach	*T2*	*T3*	*T4a*	*T4b*	*Total*
Paralateronasal	3				3 (18%)
Craniofacial	2	5	2	3	12 (60%)
Subcranial		1		3	4 (20%)
Degloving facial translocation			1		1 (2%)

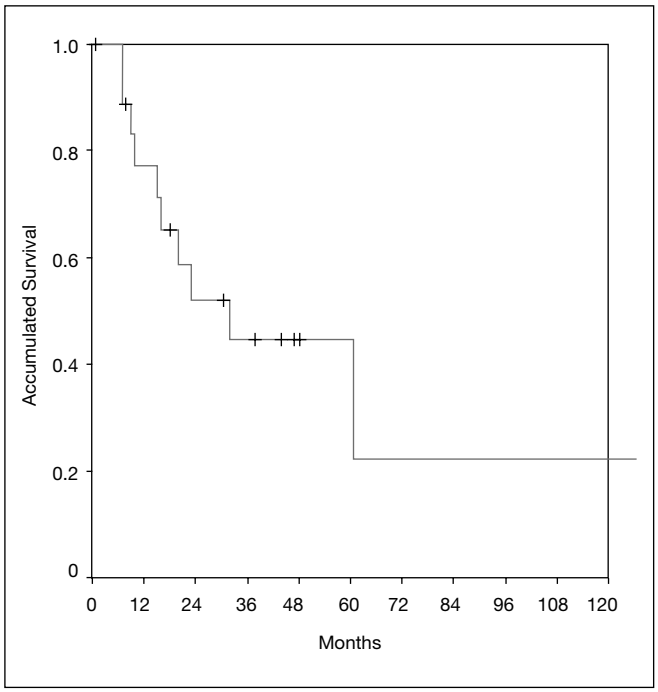

Figure 4. Overall survival of sinonasal adenocarcinomas.

Genetic

The study by MLPA showed the existence of genetic alterations in all cases studied (Table 1 and Figure 3).

In the relation between gene gains and losses and advanced T stage, only the loss of the *ABCB1* gene (*P*=.049) was significant. The rest of genetic alterations were not significant, although some genes reached close values, such as the loss of *F3* (*P*=.083), *RELA* (*P*=.063), and *LRMP* (*P*=.063), the latter for the early stages.

In relating gene gains and losses with the T stage grouped (T2-T3 vs T4a-T4b), no change was significant. Close to statistical significance were the gain of *HRAS* (*P*=.058) and loss of *LRMP* (*P*=.068) in the early stages and gain of *FGFR1* (*P*=.074) and loss of *RELA* (*P*=.098) and *BRCA1* (*P*=.074) in the advanced stages.

The relationship between genetic alterations and development of recurrence showed no significant values, although there were also some close values, such as the loss of *CREM* (*P*=.058), gain of *HRAS* (*P*=.058), loss of *RELA* (*P*=.098) and *BRCA1* (*P*=.074), the latter 2 for the absence of recurrence.

In the development of metastasis we observed significant differences in the losses of *F3* (*P*=.032) and *MIF* (*P*=.032), while the loss of *BRCA1* was close to significance (*P*=.088).

The univariate analysis between genes and survival (log rank) showed significance for the loss of *F3* (*P*=.011), BCRA1 (*P*=.0035), and *MIF* (*P*=.0429), and for the gains of *PIK3CA* (*P*=.0404) and *UTY* (*P*=.0047). The gain of *RELA* was close to significance (*P*=.0957).

In multivariate analysis genetic alterations with significance for survival were the loss of *BRCA1* (*P*=.009) (Figure 5) and *F3* (*P*=.014) (Figure 6).

DISCUSSION

The reasons which prompted this work have been on one hand, the large number of ITAC cases treated in our service and, secondly, the small number of publications relating to the molecular biology these tumours, a line in which we already have some experience.[2,7,8]

Clinical Aspects

Nineteen patients (95%) presented occupational exposure to wood dust, higher than the published data, albeit with an average time of exposure similar to ours.[2] This predominance of patients exposed to wood dust explains the absence of women, because in our medium it is unusual to have this occupation. It should be noted that the exposure for more than 20 years is associated with higher survival, but not significantly.

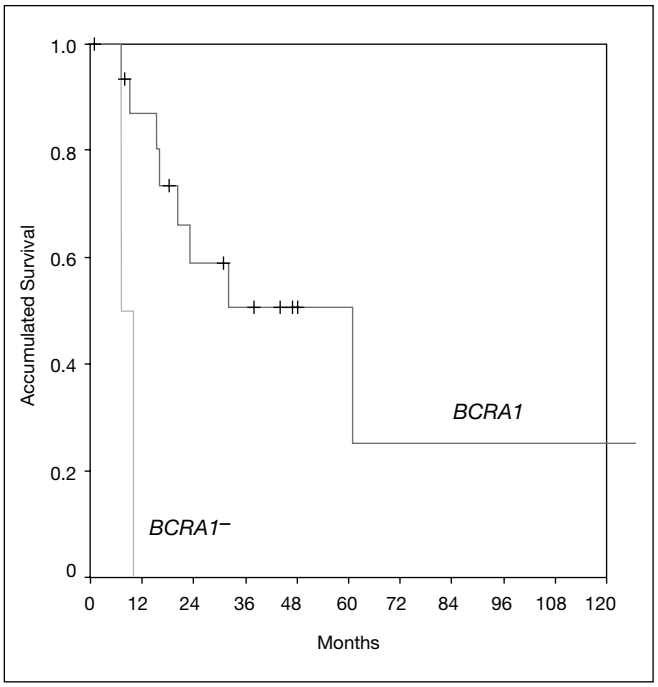

Figure 5. Kaplan-Meier curve that shows the differences in survival of patients with loss *(BCRA1⁻)* and without loss of *BCRA1* (*P*=.0035).

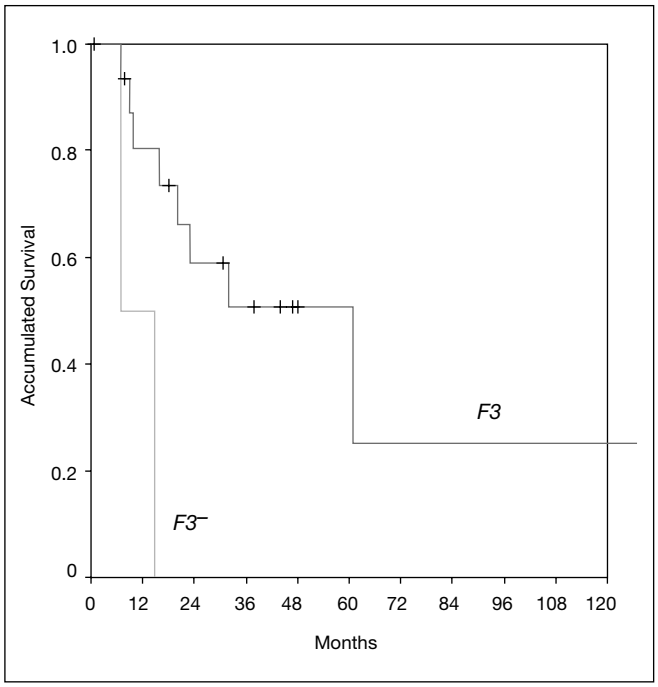

Figure 6. Kaplan-Meier curve that shows the differences in survival of patients with loss *(F3⁻)* and without loss of *F3* (*P*=.011).

The clinical presentation and extent of the tumour generally corresponded with those described in the literature.[3]

The classification used for the tumour stage was the TNM system (2002) and our findings are consistent with those of other authors, in that 80% of the cases present advanced stages (III and IV).[3] Patients with T2-3 tumours had greater survival than those with T4 (a and b) in a significant figure (*P*=.0045).

The type of treatment depended on the extent of the tumour, with surgery being predominant in mixed approaches, such as craniofacial if there is involvement of the base of the skull or subcranial when the injury affects both ethmoids, especially if there is orbital extension.

Radiation therapy was administered in a complementary manner in 70% of the cases, except for small and well defined tumours. We have not found significant differences with its use with respect to survival, probably because it was indicated in the advanced stages.

Mortality and survival are in line with what has been published; most of the deaths were by local recurrences and metastases. The overall survival at 5 years was 42%, similar to other series.[2]

Genetic Aspects

The study through MLPA showed genetic alterations in all cases studied. For exhibition purposes they will be grouped according to the chromosome in which the genes are located, trying to explain their functionality and possible involvement in the development of ITAC (Figure 2).

The *F3* gene (1p22-p21) codifies the coagulation factor III and is involved in inflammation and clotting mechanisms. We observed its loss in 10% of ITACs and its relationship with unfavourable prognosis and advanced T stage. Other authors have also seen its relationship with angiogenesis in the progression of colon adenocarcinoma.[9] These results make it reasonable to further its study in ITAC, both for its value as a marker of inflammation and for its oncogenic capacity.

The *PIK3CA* gene (3q26) encodes a protein involved in various cellular functions related to oncogenesis: cell survival and migration and intracellular vesicle transit. Forty five per cent of ITACs had losses in this gene and only 5% had gains; these were associated with worse survival, although with a limited number of cases. In a study conducted by us with CGH (comparative genomic hybridization), we observed a higher number of gains in 3q26-29 (37.5%)[8]; this discrepancy is explained by a co-hybridization of the MLPA probe with the 22q12 region.

The *IL13* (5q31) and *IL4* (5q31.1) genes encode cytokines involved in immune defence, allergy and various tumour diseases. The change in these genes appears only in the form of losses: 55% for *IL13* and 35% for *IL4*. Although they do not seem to be related to survival, they could be important in "inflammatory" processes and in the early stages of oncogenesis. These genetic alterations are consistent with the loss of chromosomic 5q arm (37.5%), detected by us using CGH in ITAC.[8]

The *ABCB1* gene (7q21.1) encodes a protein involved in membrane transport. It is linked to tumour progression and development of resistance to chemotherapy. We observed losses in 50% of ITACs and only 5% of gains, the losses being associated to the advanced T stage, but not to survival.

The *PTP4A3* gene (8q24.3) (65% gains, and 5% losses) encodes a protein involved in regulating many cellular functions, which include promoting cell growth. It has been linked to the metastasis of colon cancer and has been suggested as a therapeutic target.[10] Although metastases are not frequent events in ITAC, they should be taken into account in future studies.

The *RECQL4* gene (8q24.3) is not currently linked with adenocarcinomas, but the existence of gains in 45% could require further studies.

In general, some recent works have indicated the involvement of some gene or genes, not yet clearly identified, from the 8q24 region as prognostic factors in breast or prostate adenocarcinomas. Therefore, this region could also be considered as a target for more specific studies in ITAC.

The *CDKN2A* gene (9p21) is a tumour suppressor and is involved in controlling the cell cycle regulating the functions of CDK4 and p53. In our study 40% of ITAC showed losses and 10% gains, without relation to the survival and the stadium. This gene could play a relevant role in the early stages of ITAC.

A similar case takes place with the *LMO2* gene (11p13) (45% gains) as with the previous gene.[11]

The *HRAS* gene (11p15.5) has been involved in many tumour processes in connection with unfavourable prognosis.

In previous studies of our group,[7] it was found mutated in 16% of ITAC by a G for T transversion in the second base of codon 12. This mutation was associated with poorer survival and advanced T stadium, with no connection to previous exposure to wood dust.[7] However, other authors found no abnormalities in this gene in ITAC.[12] In this study we observed that 10% of ITAC presented gene losses and 35% gains, and the latter were significantly associated with advanced stage (T2-T3 vs T4a-T4b), but not survival.

The *EMS1* gene (11q13) (*CTTN*) codifies the cortactin protein, which regulates the operation of the cytoskeleton and intercellular unions with the extracellular matrix. We observed losses in 40% and gains in 10% of ITAC. The gene has been amplified in 20% of squamous carcinomas of the head and neck, which are associated to invasive and metastatic tumour behaviour, making it independent marker for bad prognosis.[13] Our data in ITAC is in contrast with what was observed in these tumours, where amplifications were dominant; this fact could be related to the limited number of metastases found.

The *TNFRSF7* gene (12p13) encodes a protein of the superfamily of TNF receptors with importance in cell-mediated immunity. We see gains in 50% and losses in 10%, not statistically significant in clinical evolution aspects.

The *LRMP* gene (12p12.1) encodes a protein located in the cytoplasmic side of the reticular membrane, which has not been studied in depth. We found gains in 40% of ITAC and losses in 20%. The latter were related to an almost significant extent with early tumour stages, but not to survival. Its study might be interesting in pre-invasive lesions and exposed mucous membranes.

The *RB1* gene (13q14.2) (loss in 50%) is a suppressor gene that encodes a protein involved in chromosome stability; acting on restriction points in various stages of the cell cycle. Its relationship in various tumours with advanced stages and metastasis has been observed,[14] but in our study its loss had no relationship with the prognosis or survival.

The *CDH1* gene (16q22) encodes a calcium-dependent protein from the superfamily of cadherin. Its loss of function contributes to tumour progression by activating the proliferation and development of metastasis. We observe loss of the gene in 45% of ITAC. This result is consistent with the findings of the CGH study which show that losses in the 16q21-ter region in 30% of cases.[8] We found no relationship between the altered gene and clinical-pathological and evolutionary aspects.

The *BRCA1* gene (17q21) (10% gains and 15% losses) acts as a suppressor and codifies a phosphoprotein involved in genomic stability. We observed that loss of *BRCA1* had a statistically significant relationship with both the survival and the advanced stage. The germinal mutation of this gene, together with *BCRA2*, has proved to be the most powerful predictive factor in breast and ovarian adenocarcinomas. In fact, although only 5% to 10% of breast cancers can be considered hereditary, individuals carrying a mutation in one of these genes have a 40% to 80% chance of developing breast cancer.[15] Therefore, we propose specific studies to clarify its role in ITAC.

The *BCL2* gene (18q21.3) (loss in 70%) encodes a protein that inhibits apoptosis in certain cells, such as lymphocytes. In CGH its complete loss was observed in 25% of ITAC, although it is not located in the region with more frequent losses in this chromosome: 18q22-238. Its over-expression has been observed in those adenocarcinomas with better prognosis, although it is an independent factor of poor prognosis in diffuse large cell lymphoma. It is associated with resistance to traditional chemotherapy due to stopping apoptosis. However, the number of losses reported by us would justify a more comprehensive study in ITAC.

The *MIF* gene (22q11.23) encodes a lymphokine which participates in inflammation; regulates the function of macrophages in the host defence and suppresses the anti-inflammatory effect of steroids. We observed gains in 20% and losses in 10% of ITAC; the latter were associated with worse survival, although there were few cases.

The *PDCD8* gene (Xq25-Q26) (gains in 65%) encodes a flavoprotein which is essential for breaking the nucleus of apoptotic cells. Having extensive activity in the apoptosis path it is related to other genes like BAX. However, not much is known about its direct activity in tumours. This genetic data is consistent with our CGH studies; with this technique it is the chromosome which shows a greater number of gains (58%) in ITAC.[8] Due to this, this gene is very interesting despite not reaching significant values for the survival and staging.

Finally, due to its possible significance, we want to summarize the relationships found between genetic alterations and clinical evolution aspects of ITAC (tumour stadium, recurrences, metastasis, and survival).

In reference to advanced tumour stage, we see losses in *ABCB1*, *F3*, *RELA*, and *BRCA1* and gain in *FGFR1*, although only the first alteration reaches significant values and the rest reaches close values. In the early stages, the loss of *LRMP* and gain of *HRAS* exhibited the same tendency.

The rate of recurrence was observed more in the loss of *CREM* and gain of *HRAS*, while its absence did so in losses of *RELA* and *BRCA1*, with values which were close to significance but without reaching it.

The development of metastasis was associated with statistically significant losses of *F3*, *MIF,* and *BRCA1*.

The lowest survival was also associated with loss of *F3*, BCRA1, and *MIF*, as in metastasis, and gains of *PIK3CA* and *UTY*. The same trend was noted in the gain of *RELA*, but without reaching statistical significance. In multivariate analyses, the losses in *BRCA1* and *F3* reached statistical significance.

In conclusion, the progressive knowledge of the different genetic alterations of ITAC enables us to establish its profile and serves to differentiate it, despite its histological similarity, from other frequent adenocarcinomas, such as colon adenocarcinoma. The selection of genes involved helps to establish its aetiology and its development paths, with a view to finding susceptibilities and therapeutic targets for various chemotherapeutic agents.

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