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Genomic changes of chromosomes 8p23.1 and 1q21: Novel mutations in malignant mesothelioma

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ABSTRACT

Introduction: Malignant mesothelioma is an aggressive malignancy of the thoracic cavity caused by prior asbestos exposure. In the peritoneum the mesothelioma is an extremely rare condition. In the present preliminary study, high-resolution array-comparative genomic hybridization (a-CGH) was performed to identify genetic imbalances in a series of malignant peritoneal mesothelioma cases.

Materials and methods: Between 1990 and 2008, among the cases recorded in the Apulia Mesothelioma Register, we found 22 peritoneal mesothelioma cases. CGH-array was performed on samples from all patients.

Results: The CGH-array analysis revealed multiple chromosomal imbalances. Interestingly, deletion at 8p23.1 was observed in 12 cases. Furthermore, another novel deletion at 1q21 was present in 11. Often, 1q21 and 8p23.1 losses were present in the same patient (7 cases). Losses of BAP1 and CDKN2A loci were not detected.

Discussion: The region at 8p23.1 contains the beta-defensin gene cluster (DEF) and 1q21 contains ubiquitin conjugating enzyme E2 (UBE2Q1). We hypotesized that the loss of function of ubiquitination, as well as of the defensins, could play an important role in the initial development and subsequent progression of mesothelioma.

1. Introduction

Malignant mesothelioma (MM) is a rare, lethal malignancy affecting the cells of the pleura or peritoneum surfaces, whose main known cause is occupational or environmental asbestos exposure. As in cases of exposure to other carcinogens, not all exposed individuals develop cancer even if they have inhaled high concentrations of asbestos. It has been shown that low concentrations of asbestos fibers can promote the development of mesothelioma, suggesting that a genetic predisposition (inherited or not) may play a role in mesothelioma development [1]. There is no significant evidence that surgery, chemotherapy/immunotherapy/radiotherapy treatments can significantly influence survival additionally, a trimodality approach treatment can be administered only to a limited number of patients, so the prognosis remains poor. Establishing a prognostic score in order to determine which patients are eligible for multimodal treatment remains a problem for clinicians. Numerous morphological variables seem to significantly influence the response to treatments, causing failure of the therapy. We often observe mesotheliomas which, despite presenting "low risk factors" (*i.e.* histological type, characteristics of the patient, clinical stage), rapidly progress and the therapy is ineffective. The mechanisms underlying the non-response to therapy are not yet clear. One reason could be the molecular heterogeneity of the tumor. The intra-tumor molecular heterogeneity of mesothelioma may be explained by the long

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latency and broad morphological spectrum of the neoplasm, that ranges from the epithelioid to the biphasic to the sarcomatoid type, and that within each histotype other subtypes are present (more or less frequent), in different percentages. To date, little it is yet known about the genetic events that trigger MM. Molecular changes consist of an altered expression and activation or inactivation of critical genes in oncogenesis, especially tumor suppressor genes at the 9p21 (INK4) and 22q12 (NF2) loci. Also, BAP1 mutations have been identified in familial and sporadic mesotheliomas, so it could be hypothesized that germline BAP1 mutations may contribute to the susceptibility to asbestos-related mesothelioma through a mechanism involving gene-environment interaction [2]. In fact, tumor heterogeneity is not only a characteristic of the tumor within the same patient but is also influenced by the microenvironment. This is what pathologists observe when p16/CDKN2A or BAP1 or Ki67 are evaluated by immunohistochemistry or FISH methods. The tumor cells show different molecular imbalances in different areas, so they could respond to therapy differently. Krismann when examining different histological areas of the same tumor observed different alterations (losses or gains) of chromosomal regions, while in histologically similar areas the losses were different whereas the amplifications were identical [3].

What are the frequent and/or rare "driver mutations" in mesothelioma? In a morphologically heterogeneous cancer, whose incidence is increasing, the identification of these alterations is difficult but absolutely essential to be able to devise personalized therapy. This is the challenge of the future. Most studies have been focused on pleural mesothelioma, while peritoneal mesothelioma is still considered a very rare neoplasm, with a history of asbestos exposure that is not always well documented. Peritoneal mesothelioma was first described in 1908 by Miller and Wynn. It is characterized by rapid fatal course with a median survival of 6–12 months. To date, it is not clear whether mesothelioma forms in different sites, which present the same histology and risk factors, actually do share genomic alterations or undergo similar oncogenic transformations. In the present preliminary study, high-resolution array-comparative genomic hybridization (a-CGH) was performed to identify genetic imbalances in a series of malignant peritoneal mesothelioma cases.

2. Materials and methods

Between 1990 and 2008, among the cases recorded in the Apulia Mesothelioma Register, we found 22 peritoneal mesothelioma cases, mostly arising in patients with occupational/environmental or domestic (cohabitant son/daughter or wife) asbestos exposure. Demographic data, including gender, age, age at first asbestos exposure, occupational history, domestic exposure based on the characteristics of the dwellings, namely the presence or use of asbestos-containing materials at home or by a cohabitant person, duration of the exposure (the difference between the start and the end data), and personal and family health history, were collected and archived from the questionnaire/interview employing National Mesothelioma Register Guidelines standard criteria. All patients lived in Apulia (southern Italy), in the city of Bari, where asbestos was actively produced at the Fibronit factory [4]. The study was approved by the local Ethics Committee of the Policlinico-Hospital, Bari, Italy (accession number 5062, June 22, 2016). All histological slides were reviewed by expert pathologists (GS and AM) and tumors were classified according to WHO [5] criteria. Tumor tissues were fixed in 4% buffered formaldehyde and paraffin-embedded according to standard histopathologic methods. All diagnoses were supported by immunohistochemistry (IHC) according to current guidelines [5]. Table 1A and Table 1B show the clinical information of the cases analyzed. At diagnosis patients ranged from 36 to 80 years old of age (mean 61.6 years), asbestos exposure was well documented in 18/22 cases and an occupational asbestos history was present in 10 of them (mean exposure 16.5 years). Patients (7 cases) with environmental asbestos exposure lived near the polluted sites and in one case the exposure was domestic. Fourteen tumors were epithelioid, five biphasic and three sarcomatoid types. Array-comparative genomic hybridization (a-CGH) was performed on samples from all patients to identify those genes related to tumor development and progression. The CGH-array technique is described in our previous works [6,7].

Validation of genomic changes by fluorescence in situ hybridization (FISH): paraffin embedded tissue sections after pretreatment were hybridizated using BAC probes RP11-161B1 (8p23.1) and RP11-307C12 (1q21) (Empire Genomics). This probes gave only one hybridization signal on each chromosome. FISH was performed according to the technique described by web site https://www.empiregenomics.com/.

Also, FISH locus-specific CDKN2A (9p21) probe (Abbott, Abbott Park, IL) to detect choromosome 9 deletion and immunohistochemistry for BAP1 (3p21) (C4, Santa Cruz Biotechnology, Santa Cruz, USA) were carried out in one representive case (case 13, epithelioid mesotelioma group).

Vysis LSI CDKN2A/CEP 9 probes are provided in one vial as a mixture of the LSI CDKN2A (p16) probe labeled with Spectrum Orange and the CEP 9 probe labeled with Spectrum Green. The LSI CDKN2A probe spans approximately 222 kb and contains a number of genetic loci including D9S1749, DS1747, p16 (INK4B), p14 (ARF), D9S1748, p15(INK4B), and D9S1752. The CEP 9 Spectrum Green probe hybridizes to alpha satellite sequences specific to chromosome 9, CE Marked. At least 100 cells were scored for each case. Our institution used a cutoff more than 20 percent to define a homozygous deletion.

3. Results

The CGH-array analysis revealed multiple chromosomal imbalances (Tab. 1 A–B). Deletions were less frequent than gains. Interestingly, deletion at 8p23.1 was observed in 12 cases, 10 of them with exposure to asbestos. Furthermore, another novel deletion at 1q21 was present in 11 cases and only one of them did not have a documented history of asbestos exposure. Often, 1q21 and 8p23.1 losses were present in the same patient (7 cases). The BAP1 immnuhistochemistry, performed in case 7 of bi-phasic and sarcomatoid peritoneal mesothelioma group (Fig. 1a), showed a strong positivity in neoplastic nuclei (Fig. 1b). In the same case the CGH-array results (Fig. 1e) were confirmed by FISH analysis which showed normal CDKN2A gene expression (Fig. 1c) and a homozygous deletion of 8p23.1 (Fig. 1d).

4. Discussion

Although a number of studies have been conducted to evaluate genetic events associated with the development and progression of MM, the first molecular mechanism implicated in the initiation of genomic instability remains obscure, and the identification of predictive markers is crucial. In particular, the identification of genes that are altered in this malignancy could be valuable, indicating potential therapeutic targets or prognostic indicators. For clinicians the goal is to identify genomic markers helping to detect the genes eliciting that cancer's therapeutic susceptibility. Within each histological type of mesothelioma, we often observe morphological patterns in different percentages. No morphological grading-system serving for an initial and possible stratification of patients to be submitted to different therapeutic treatments has yet been standardized. This may explain therapeutic failure but it also stresses the concept of intra-tumoral heterogeneity and the need for multi-instututional studies to identify genes related to therapy and survival.

The region at 8p23.1 contains the beta-defensin gene cluster (DEF). Defensins (α-defensin or ß-defensin) are produced in the respiratory, gastrointestinal, genitourinary tract, skin and blood cells. They are considered a first line of defense against invading pathogens (antimicrobial, chemotactic and regulatory functions). The fact that ß-defensins are expressed in most epithelial cells and are impaired in many inflammatory diseases vindicates the assumption that defensins are

Table 1A
Summary of clinical and molecular cytogenetic data (a-CGH) of patients affected by malignant peritoneal mesothelioma, epithelioid type.

Case n. (gender)	Age (yrs)	Type	Asbestos exposure	Survival (months)	Losses	Gains
1 ♂	56	Epitheliod	Occupational (1961-1973)	14†	**8p23.1,** 9p12→p11, 9q12→q21.33	1p36.33→p36.32, 3p25.3→p25.1, 3p22.2→p22.1, 3q29, 4p16.3→p16.1, 4q13.1, 5p15.33, 7p22.3→p22.1, 8q24.3, 9q34.11→q34.3,10q26.3, 11p15.5→p15.4, 11q13.1→q13.2, 11q13.3→q13.4, 12q24.33, 13q12.2, 13q34, 16q22.1→q22.2, 16q24.1→q24.3, 17q24.3→q25.3, 19p13.3, 20q13.33, 22q11.21→q13.23, 22q13.2, Xp22.33, Xq22.2, Xq28
2 ♂	62	Epitheliod	Occupational (1952-1961)	6†	***1q21***, 2q11.1→q13, 6q27, **8p23.1,** 9p12→p11, 9q12→q21.33, 9q21.33→q33.1, 17p12→p11.	1p36.33→36.32, 1q31, 3p25.3→p25.1, 3p22.2→p22.1, 3q29, 4q13.1, 4p16.3→p16.1, 4q13.1, 5p15.33, 7p22.3→p22.1, 7q21.11, 8q24.3, 9q34.3, 10q26.3, 11p15.5→p15.4, 13q34, 16q22.1→22.2, 16q24.3, 17p13.1, 17q25.3, 19p13.3, 20q13.33, 21q22.3, 22q11.21→q11.23, Xp22.33, Xq22.2, Xq28
3 ♂	54	Epitheliod	Environmental	5†	**8p23.1**, 9p12→p11, 9q12→q21.33, 9q21.33→q33.1, 10p13→p12.32	1p36.33→p36.32, 3p25.3→p25.1, 3p22.2→p22.1, 3q29, 4p16.3→p16.1, 4q13.1, 5p15.33, 7p22.3→p22.1, 7q21.11, 8q24.3, 9q34.11→q34.3, 10q26.3, 11p15.5→p15.4, 11q13.1→q13.2, 12q24.33, 13q12.2, 13q34, 16q24.1→q24.2, 17q24.3→q25.3, 19p13.3, 20p13, 20q13.33, Xp22.33, Xq28
4 ♂	75	Epitheliod	Occupational (1958-1960)	4†	**8p23.1**	1p36.33→36.32, 1q31, 3p25.3→p25.1, 3q29, 4p16.3→p16.1, 4q13.1, 5p15.33, 7p22.3→22.1, 7q21.11, 8q24.3, 9q34.11→q34.3, 10q26.3, 11p15.5→p15.4, 11q13.1→q13.2, 11q13.3, 12q24.33, 13q12.2, 13q33.2, 13q34, 16q22.1→q22.2, 16q24.1→q24.2, 17p13.1, 17q24.3→q25.3, 19p13.3, 20p13, 20q13.33, 21q22.3, 22q11.21→q11.23, Xp22.33, Xq22.2, Xq28
5 ♂	62	Epitheliod	Occupational (1956-1979)	2†	***1q21***, 2q11.1→q13, 6q27, **8p23.1**, 9q12→q21.33, 9q21.33→q33.1	1p36.33→p36.32, 1q31, 3q29, 4p16.3→p16.1, 4q13.1, 5p15.33, 7p22.3→22.1, 7q21.11, 8q24.3, 9q34.11→q34.3, 10q26.3, 11p15.5→p15.4, 11q13.1→q13.2, 11q13.3→q13.4, 12q24.33, 13q12.2, 13q33.2, 16q22.1→q22.2, 17q24.3→q25.3, 19p13.3, 20p13, Xp22.33, Xq22.2, Xq28
6 ♂ *	36	Epitheliod	Environmental	208§ last follow-up April 2018	***1q21***, 2q11.1→q13, **8p23.1**, 9p12→p11, 9q21.33→q33.1, 9q12→q21.33, 17p12→p11.2	1p36.33→p36.32, 1q31, 3p25.3→p25.1, 3p22.2→p22.1, 3q29, 4p16.3→p16.1, 4q13.1, 5p15.33, 7p22.3→p22.1, 7q21.11, 8q24.3, 9q34.11→q34.3, 10q26.3, 11q13.1→q13.2, 11p15.5→p15.4, 11q13.3→q13.4, 12q24.33, 13q33.2, 16q22.1→q22.2, 17p13.1, 17q24.3→q25.3, 19p13.3, 20p13→p12.3, 22q11.21→q11.23, Xp22.33, Xq22.2, Xq28
7 ♂ ** Patient n.1	71	Epitheliod	NR	27†	1p32.3→p31.2, 1p31.1→p21.3, ***1p13.3→q23.3,*** 1q25.3, 1q32.1→q41, 1q41, 2p12→q13, 6p25.2→p24.3, 6q25.3→q26, 6q26, 6q26, 6q27, **8p23.1**, 9p24.3→p24.1, 9q21.31→q22.32, 9q31.1→9q33.3, 10p15.3,10p14→p12.31, 10q22.1→q23.31, 11p13, 11q13.2, 12q24.13, 13q14.2, 13q21.1→q21.2, 15q25.3→q26.1, 17p12→p11.2, 17q11.2→q12, 18q22.2, 18q22.3→q23, 19p13.3, 19p13.2, 19p12, 19q13.42, 19q13.43, 20p13→p12.2, 20p11.23→q11.21, 20q11.23→q13.12, 21q22.11, 21q22.3, 22q11.23→q12.1, 22q12.3, 22q13.2	1p36.33, 1p31.1, 1p21.3, 3p21.31, 3q29, 4p16.3→p16.1, 4q35.2, 5p15.33, 5q34→q35.3, 6p24.3→p24.2, 6q26, 7p22.3, 7p22.3→p22.1, 7q11.23, 9q33.3→q34.3, 10p15.1→p14, 10p12.31, 10q26.13, 11p15.5, 11p15.5→p15.4, 11q12.2→q13.2, 11q13.3, 12q24.23→q24.31, 12q24.33, 13q21.1, 14q21.1, 14q32.32→q32.33, 16p13.3, 16q22.1, 16q24.2, 16q24.3, 17p13.3, 17p13.3→p13.1, 17p11.2, 17q12, 17q21.2→q21.31, 17q25.1, 17q25.2→q25.3, 18p11. 31, 18q22.1, 18q22.3, 18q23, 19p13.3, 19p13.2, 19p13.2→p13.12, 19p13.11, 19q13.11→q13.2, 19q13.31→q13.42, 19q13.43, 20q11.21, 20q13.33, 21q21.1→q21.3, 21q22.2, 21q22.3, 22q11.1→q11.23, 22q13.1→q13.2, 22q13.31, 22q13.33, Xp21.2→p11.23, Xq28,Yq11.22
8 ♂	68	Epitheliod	Environmental	18†	***1q21***, 2q11.1→q13, 6q27, **8p23.1**, 9q12→q21.33, 17p12→p11.2	1p36.33→p36.32, 3q29, 4p16.3, 5p15.33, 7p22.3→p22.1, 9q34.11→q34.3, 11p15.5, 11q13.3→q13.4, 12q24.33, 16q22.1→q22.2, 17q24.3→q25.3, 19p13.3, 22q11.21→q11.23, Xq28
9 ♂	68	Epithelioid	Occupational (1958-1989)	46†	***1q21***, 2q11.1→q13, 9p12→p11, 9q12→q31.3	1p36.33→p36.32, 5p15.33, 17p13.1, 22q11.21→q11.23
10 ♂	71	Epithelioid	NR	25†	2q11.1→q13, 6q27	1p36.33, 3q29, 5p15.33, 7p22.3 →p22.1, 11q13.1→q13.2, 12q24.33, Xq28
11♂	50	Epithelioid	Environmental	28†	***1q21***	**1p36.33**→p36.32, 4p16.1, 10q26.3, 11p15.5→p15.4, Xp22.33, Xq28
12 ♂	51	Epithelioid	Environmental	4†	***1q21***	**1p36.33**→p36.32, 1q31, 3p22.2→p22.1,3q29, 4p16.3→q13.1, 5p15.33, 7p22.3→p22.1, 7q21.11, 8q24.3, 9q34.11→q34.33, 10q26.3, 11p15.5→p15.4, 11q13.1→q13.2, 11q13.3→q13.4, 12q24.33, 13q33.2, 19p13.3, 20p13, Xq22.2, Xq28
13 ♂	58	Epithelioid	Occupational (1935-1967)	17†	***1q21***, 2q11.1→q13, 9p12→p11, 9q12→q33.3, 17p12→p11.2	**1p36.33**→p36.32, 4p16.3→p16.1, 5p15.33, 8q24.3, 9q34.3, 10q26.3, 11p15.5→p15.4, 11q13.1→q13.2, 11q13.3→q13.4, 19p13.3, Xq28
14 ♂ *** Patient n.2	82	Epithelioid	NR	63†	1p13.1→q21.1, 2p11.2→q13, 2q33.1, 5q23.1, 6p25.3→p25.1, 6q25.3, 6q26 →q27, **8p23.1**, 9p12→q13, 11q13.2, 12q24.13, 13q14.2, 13q21.2, 16q24.1, 17q11.2→q12, 19q13.42→q13.43, 20p12.3, 20p11.21→q11.1, 21q22.11, 21q22.2→q22.3, 22q11.23→q12.1, 22q12.2→q12.3	1p31.1, 2q33.1, 3q29, 4p16.3, 4p16.1, 5q13.2, 5q35.1→q35.3, 6q26, 7p22.3, 7p22.1, 7q11.23, 7q22.1, 10p13,10p12.31,11p15.5→p15.4, 11q13.1→q13.2, 11q13.3→q13.5, 12q24.23→q24.31, 13q21.1, 13q34, 15q24.3→q25.1, 15q26.1, 16p13.3, 16q24.2, 16q24.3, 17p13.3→p13.1, 17p11.2, 17q12→q21.31, 17q25.1, 17q25.1→q25.2, 17q25.3, 18p11.32→p11.31, 18q21.33→q22.1, 18q23, 19p13.3→p13.2, 19p13.2, 19p13.2→p13.11, 19q13.11→q13.42, 19q13.43, 20q13.33, 21q11.2, 21q21.1→q21.3, 21q22.2, 21q22.3, 22q11.21, 22q11.21→q11.23, 22q12.2, 22q13.1→q13.2, Xp21.2→p11.23, Yq11.22.1

Abbreviations: NR not reported; patient status: †deceased; §alive. In bolt type losses at 1q21 and 8p23.1. 1q21 and 8p23.1.
*Serio G et al. Int J Mol Sci 2017, 18(8):1818 online, **Serio G et al. Pathol Int 2009, 59:415-421, ***Serio G et al. Int J Clin Exp Pathol 2016, 9(7): 7658-7667.

Table 1B
Summary of clinical and molecular cytogenetic data (a-CGH) of patients affected by malignant peritoneal mesothelioma, biphasic and sarcomatoid type.

Case n. (gender)	Age (yrs)	Histotype	Asbestos exposure	Survival (months)	Losses	Gains
1 ♂	65	Biphasic	Environmental	2†	***1q21***, 2q11.1→q13, 6q27, **8p23.1**, 9q12→q21.33, 9q21.33→q33.1	4p16.3→p16.1, 7q21.11, 10q26.3, 19p13.3, Xp22.33
2 ♂	80	Sarcomatoid	Occupational (1945-1953)	4†	1q11.1→q13, 5p15.33ter, 6q27, **8p23.1**, 9q12→q21.33, 17p12→p11.2	3q29, 5p15.33
3♂	58	Biphasic	Occupational (1968-1972)	2†	–	1p36.33→p36.32, 5p15.33, 9q34.11→q34.3, 10q26.3, 11p15.5→p15.4, 19p13.3, Xq28
4 ♀	60	Biphasic	NR	2†	9q12→q21.33, 9q21.33→q33.1	1p36.33→p36.32, 1q31, 2p16.3, 3p25.3→p25.1, 3p22.2→p22.1, 3q29, 4p16.3→p16.1, 5p15.33, 7p22.3→p22.1, 7q21.11, 8q24.3, 9q34.11→q34.3, 10q26.3, 11p15.5→p15.4, 11q13.1→q13.2, 11q13.3→q13.4, 13q33.2, 16q22.1→q22.2, 17p13.1, 17q24.3→q25.3, 19p13.3, 20p13, 22q11.21→q11.23, Xp22.33, Xq28
5♀	58	Sarcomatoid	Environmental	8†	–	1p36.33→p36.32, 5p15.33, 17q24.3→q25.3, 20p13
6 ♂	67	Sarcomatoid	Occupational (1952-1985)	2†	18q23.3	4q28.3→4q31.1, 12q22, 20p12.2, Xq13.1
7 ♂	52	Biphasic	Occupational (1948-1976)	10†	1p36.33→1p34.3, 1p34.3→1p32.3, 1p32.3→1p31.1, 1p22.2→1p21.3, 1p21.1→***1q21.3***, 1q32.2, 2q24.1, 6p24.2→6p22.1, 6p12.3→6q14.3, 6q16.2→6q25.2, 7q11.22, **8p23.1**→8p22, 8p22→8p21.2, 9p24.2→9p24.1, 10p15.1→10q23.31, 10q25.2→10q26.13, 11p15.2→11p13, 11q13.2, 11q14.3, 17p13.1→17p11.2, 18q22.1→18q22.3, 19p13.3, 19p12→19q12, 19q13.43, 20p13→20p12.2, 20p11.21, 20q11.23, 20q12, 21q22.3, 22q11.23→22q12.1, 22q12.2→22q13.2, Xp11.21→Xq12, Xq12	1p31.1, 1p21.3, 1q22→1q23.3, 2q37.1, 3p21.31, 3q26.1→3q27.3, 4p15.1, 4q28.1→4q28.3, 5q15→5q21.3, 7q22.1, 8p22, 9p21.1→9p13.1, 11q12.2→11q13.2, 11q13.2→11q13.4, 12q13.11→12q13.13, 15q26.1, 16q22.1, 18q21.31→18q22.1, 19p13.3, 19p13.2→19p12, 19q13.12, 21q21.1→21q21.2, 22q11.21→22q11.23, 22q13.2, Xp21.3
8♀	72	Biphasic	Domestic (1952-1963)	2†	1p21.3→1p13.3, 2p16.3, 3q29, 4q13.2, 6p22.1, 6p21.32, 13q14.2	1p21.3, 2p21→2p16.3, 6p21.32→6p21.2, 11p12→11q13.5, 12q13.11→12q13.12, 17p11.2, 19q13.2→19q13.33, 20q11.21

Abbreviations: NR, not reported; patient status: †,deceased; §, alive. In bolt type losses at 1q21 and 8p23.1.

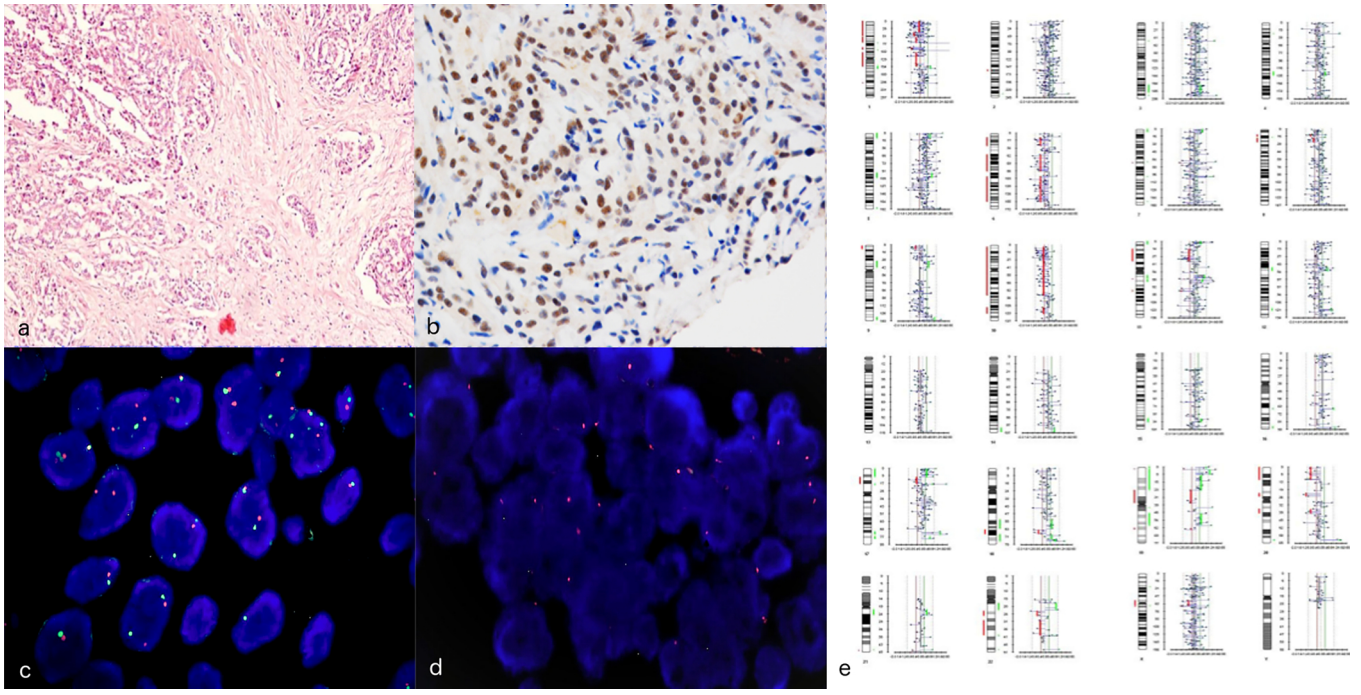

Fig. 1. CASE 7: epithelioid growth pattern of biphasic peritoneal mesothelioma (a. haematoxylin and eosin, original magnification x 100); the neoplastic nuclei showed a strong positivity for BAP1 (b. original magnification x 200); FISH analysis shows the CDKN2A (p16) normal expression (c. presence of 2 red and green signals) and a homozygous 8p23.1 deletion (d. loss of 2 red signal per cells); CGH-array: losses at 1q21 and 8p23.1 are detected (e).

involved in the pathogenesis of inflammatory processes and also in many cancers (pancreas, lung, breast, etc) [8]. Asbestos causes DNA damage directly, by mechanically interfering with the segregation of chromosomes during mitosis, and indirectly by inducing mesothelial cells and macrophages to release mutagenic reactive oxygen and nitrogen species. Since malignant mesothelioma of the peritoneum has been observed in individuals with recurrent peritonitis (diverticulitis, Crohn's disease, Familial Mediterranean fever etc.) the chronic serosal inflammation might contribute to trigger mesothelioma. In a recent report, Sneddon et al [9] report a high percentage of deletion at 8p23.1 in cell lines derived from tumor mesothelioma cells found in pleural effusion samples using whole exome and transcriptome sequencing methods. Employing the whole-genome arrayCGH strategy, we successfully identified a high total number of chromosomal aberrations in a set of peritoneal asbestos-related mesothelioma. Interestingly, loss at 8p23.1 was more frequent. Our results suggest a common mechanism of asbestos-induced neoplastic transformation in both pleural and peritoneal mesothelioma, and as suggested by Sneddon et collegues (9), analysis of the fluids (peritoneum or pleura) can permit a rapid molecular evaluation to detect predictive prognostic marker for personalized therapy purposes.

The region at 1q21 contains ubiquitin E2 conjugating enzyme (UBE2Q1), a gene that plays an important role in the conjugation of proteins and their degradation. Over the years, ubiquitin has been clarified to play a role not only as a protein marker but also to have other functions, such as directing the transport of proteins into and out of the cell. By connecting multiple ubiquitins together in short or long chains, or using different connections between the molecules, very different signals can be encoded. Numerous cellular processes are regulated by ubiquitination, mediated by growth factors, and potentially affect all aspects of a cell life. Consequently, there are also many signal transmission pathways that, in cases of alteration of this mechanism, may be seriously compromised, also giving rise to various types of cancer (ie breast and ovarian cancersin cases of BRCA1 mutations) [10]. Our results show a loss of function of the UBE2Q1 gene in 10 patients with long lasting asbestos exposure (occupational/environmental).

In our study BAP1 and CDKN2A losses were not detected, suggesting differences for this genes-site in pleural and peritoneal mesotheliomas. Loss at 8p23.1 in asbestos-related mesothelioma of the pleura and peritoneum and the absence of prognostic biomarkers so far considered to be the most common (CDKN2A, BAP1) stress the need for a homogeneous morphological prognostic grading-system, confirm the existence of a high degree of tumor genetic heterogeneity, and highlight the importance of detecting rare driver mutations that, in a morphologically complex cancer, might be useful to devise personalized therapies.

In conclusion, in our preliminary study, we may hypothesize that the loss of function of ubiquitination, as well as of the defensins, could play an important role in the initial development and subsequent progression of mesothelioma. These results need further confirmation with more sensitive and specific molecular analysis (DNA sequencing and FISH analysis) focused on 8p23.1 and 1q21 changes. The status of BAP1 and CDKN2A genes will be investigated with immunohistochemistry and FISH in all cases, as we perfomed the case 7. Furthermore the results will be compared with a large cohort of pleural mesothelioma. These represent the objectives of our study and will be a subject of upcoming report.

Conflict of interest

None has any conflict of interest.

Authors' contributions

All authors contributed to drafting and reviewing of the manuscript.

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