Clinicopathological significance of mitochondrial D-Loop mutations in head and neck carcinoma

A Lièvre1, H Blons1,2, AM Houllier1, O Laccourreye3, D Brasnu3, P Beaune1,2 and P Laurent-Puig*,1,2

1INSERM, U490, Université René Descartes, Paris F-75006, France; 2Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, pôle biologie, Paris F-75015, France; 3Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, service d’Oto-Rhino-Laryngologie et de Chirurgie cervico-faciale, Paris F-75015, France

Mitochondrial DNA mutations have been reported in several types of tumours, including head and neck squamous cell carcinoma (HNSCC). The noncoding region of the Displacement-Loop (D-Loop) has emerged as a mutational hotspot and we recently found that they were associated with prognosis and response to 5 fluorouracil (5FU) in colon cancers. In order to evaluate the frequency of D-Loop mutations in a large series of HNSCC and establish correlations with clinicopathologic parameters, we sequenced the D-Loop of 109 HNSCC before a treatment by neoadjuvant 5FU-cisplatin-based chemotherapy and surgery. Then, we correlated these mutations with prognosis and response to chemotherapy. A D-Loop mutation was identified in 21% of the tumors, the majority of them were located in a C-tract (D310). The prevalence of D310 mutations increased significantly with the number of cytosines in the matched normal tissue sequence (P = 0.02). Hypopharyngeal cancer was significantly more frequent (P = 0.03) and tobacco consumption more important (P = 0.01) in the group of patients with D-Loop mutation. The presence of D-Loop mutation was not associated with prognosis or with response to neoadjuvant chemotherapy. These results suggest that D-Loop mutations should be considered as a cancer biomarker that may be useful for the early detection of HNSCC in individuals at risk of this cancer.

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Although much knowledge has been collected concerning alterations in cancer cell nuclear DNA (nDNA), less attention has been paid to mutations within mitochondrial DNA (mtDNA). Mitochondrial DNA is a 16 569 bp double-stranded, circular DNA encoding 13 respiratory chain protein subunits, 22 tRNAs and two rRNAs. It is also composed of a 1.2 kb noncoding region, the Displacement-Loop (D-Loop), which contains essential transcription and replication elements. Owing to a particular susceptibility to oxidative damage due to high levels of reactive oxygen species (ROS) generation in mitochondria, inefficient DNA repair system and a lack of protective histones in this organelle, mutation rate has been reported to be 10–17-fold higher in the mtDNA than in the nDNA (Fliss et al, 2000). Mitochondria has long been suspected to be involved in carcinogenesis (Warburg, 1956). Its role in apoptosis supports this hypothesis (Zamzami and Kroemer, 2001) and it was shown that mtDNA mutations may lead to a dysregulation of oxidative phosphorylation that can enhance production of the carcinogenic ROS. Over last years, somatic mtDNA mutations have been reported in many human tumours (Polyak et al, 1998; Maximo et al, 2000; Richard et al, 2000; Yeh et al, 2000; Hibi et al, 2001b; Sanchez-Cespedes et al, 2001; Kirches et al, 2001; Liu et al, 2001; Lièvre et al, 2005), including head and neck squamous cell carcinoma (HNSCC) that were found mutated in small series in 37–77% of the cases (Fliss et al, 2000; Sanchez-Cespedes et al, 2001; Ha et al, 2002; Tan et al, 2003; Poetsch et al, 2004). Although mutations may occur throughout the mitochondrial genome, the vast majority of them have been described in the noncoding region of the D-Loop and particularly in a mononucleotide repeat named D310 (C-tract, nucleotide position: 303–315) that has emerged as a mutational hotspot in HNSCC (Fliss et al, 2000; Sanchez-Cespedes et al, 2001; Ha et al, 2002; Tan et al, 2003; Poetsch et al, 2004).

Head and neck squamous cell carcinoma represents 5% of all newly diagnosed cancer cases in the northern and western Europe and in the US (Muir and Weiland, 1995) where it represents a public health problem. As in most solid tumours, head and neck tumor epithelial cells undergo nuclear genetic alterations in proto-oncogenes and tumour suppressor genes through a multistep process. One of the most frequent alterations are TP53 somatic mutations found in more than a half of the cases. Allelic losses are also frequently observed on 3p, 9p and 17p (Nawroz et al, 1994; van der Riet et al, 1994; Blons et al, 1999), as the amplification of the cyclin D1 oncogene. Identification of new genetic alterations associated with HNSCC is important since they may allow to better understand the molecular mechanisms involved in head and neck carcinogenesis and serve as a molecular marker that may be used in evaluating the tumorigenic potential of head and neck lesions in individuals at high risk of cancer. Recently, we found that D-Loop...
Mutations were linked to prognosis and lack of benefit from 5 fluorouracil (5FU)-based adjuvant chemotherapy in colorectal carcinomas (Lievre et al, 2005). Neoadjuvant chemotherapy have been recently developed in new treatment strategies of locally advanced HNSCC and has been shown to be curative in complete clinical responder patients with a cancer of the pharyngolarynx (Laccourreye et al, 2001). Identification of molecular factors associated with response to neoadjuvant chemotherapy has become an important goal because it may help to select patients who could benefit from this treatment and so from a possible organ preservation. Therefore, the aims of this work were to determine the frequency of D-Loop mutations in a large series of HNSCC, establish correlations between D-Loop mutations and clinicopathologic parameters and determine the impact of these mutations on prognosis and response to neoadjuvant 5FU–cisplatin-based chemotherapy in HNSCC patients.

MATERIALS AND METHODS

Patients

This study was performed on patients with histologically proven HNSCC managed at the Laennec Hospital (Paris, France) who had been prospectively included in a previous study in which response to neoadjuvant chemotherapy was assessed (Blons et al, 2004). The inclusion criteria retained were the following: no previous history of cancer, no multiple tumour locations, no contraindication for a 5FU- or cisplatin-based chemotherapy and indication for neoadjuvant chemotherapy prior to surgery or radiotherapy. This work was performed according to the French Law and blood samples and tumour biopsies were obtained after written informed consent and approval of the local ethic committee (CPPRB-496.017). Among the 148 patients initially included, 109 (98 male and 11 female subjects, mean age: 57.8 ± 7.4 years) for whom DNA was still available were screened for mtDNA mutations. Tumours were located in the oral cavity (n = 13), the oropharynx (n = 46), the hypopharynx (n = 27) and the endolarynx (n = 25). They were classified according to the TNM classification and staged as recommended by the American Joint Committee on Cancer. There were five T1, 42 T2, 28 T3, 34 T4 tumours, and 49 tumours were N0 whereas 60 of them were N+. Three tumours were stage I, 23 were stage II, 25 were stage III and 58 were stage IV. Among the patients, 63 smoked >35 pack-years; 34, 15–35 pack-years and 12, <15 pack-years. A TP53 mutation was present in 72 patients (67.3%). Clinicopathologic characteristics of the patients are listed in Table 1. All patients received a neoadjuvant chemotherapy before surgery or radiotherapy that consisted of cisplatin (25 mg/m² day) and 5FU (1 g/m² day) delivered as a daily continuous i.v. dose in 4-day courses. Three courses were repeated at 16–21 days intervals. Clinical response was assessed as defined by the Eastern Cooperative Oncology Group. Responder patients (R) were defined by patients who showed at least a 50% decrease in tumour size. In this series, 72 patients (66.1%) were responders and 37 (33.9%) were nonresponders.

Table 1 Clinicopathologic characteristics of HNSCC patients according to D-Loop mutation

| Clinicopathologic characteristics | Number of patients | Patients with mutated tumour (%) | Patients with nonmutated tumour (%) | P-value |
|----------------------------------|-------------------|---------------------------------|-----------------------------------|---------|
| Age (mean)                       | 56.9 ± 2          | 58.0 ± 1                        | 0.655                             |
| Gender                           |                   |                                 |                                   |         |
| Male                             | 98 (89.9)         | 22 (95.6)                       | 76 (88.4)                         | 0.3     |
| Female                           | 11 (10.1)         | 1 (4.4)                         | 10 (11.6)                         |         |
| Tumour sites                     |                   |                                 |                                   |         |
| Oral cavity                      | 13 (11.9)         | 1 (4.4)                         | 12 (13.9)                         | 0.03    |
| Oropharynx                       | 46 (42.2)         | 7 (30.4)                        | 39 (45.4)                         |         |
| Hypopharynx                      | 27 (24.8)         | 11 (47.8)                       | 16 (18.6)                         |         |
| Endolarynx                       | 23 (21.1)         | 4 (17.4)                        | 19 (22.1)                         |         |
| Tumour stage                     |                   |                                 |                                   |         |
| I                                | 3 (2.8)           | 2 (87)                          | 1 (12)                            | 0.24    |
| II                               | 23 (21.1)         | 4 (17.4)                        | 19 (22.1)                         |         |
| III                              | 25 (22.9)         | 6 (26.1)                        | 19 (22.1)                         |         |
| IV                               | 58 (53.2)         | 11 (47.8)                       | 47 (54.6)                         |         |
| Tobacco (mean pack-year)         | 48 ± 3            | 37 ± 2                          | 0.01                              |
| TP53 mutation                    |                   |                                 |                                   |         |
| Yes                              | 72 (67.3)         | 19 (82.6)                       | 53 (63.1)                         | 0.07    |
| No                               | 35 (32.7)         | 4 (17.4)                        | 31 (36.9)                         |         |
| Response to chemotherapy         |                   |                                 |                                   |         |
| Responder                        | 72 (66.1)         | 14 (60.9)                       | 58 (67.4)                         | 0.55    |
| Nonresponder                     | 37 (33.9)         | 9 (39.1)                        | 28 (32.6)                         |         |
| Total                            | 109 (100)         | 23                               | 86                                |         |

HNSCC = head and neck squamous cell carcinoma; D-Loop = Displacement-Loop.

Foster City, CA, USA) using primers F47: 5'-GCG AGC GAC TAC AAC CAC GAC-3' (forward) and R15: 5'-CTG GGG GTG TCT TTG GG (reverse) as described previously (Lievre et al, 2005). The 2467 bp PCR products (nucleotide positions: 14679 – 577) were then purified using G-50 Sephadex superfine (Amersham Biosciences, Orsay, France) on Multiscreen support (Millipore, Bedford, MA, USA).

Direct sequencing of the D310 repeat

The D310 repeat sequencing was performed on a Gene Amp PCR System 9700 (Applied Biosystems) using a Big Dye Terminator cycle sequencing kit (Applied Biosystems) as described previously (Lievre et al, 2005). Sequences were analysed on an ABI Prism® 3700 DNA Analyser automated sequencer (Applied Biosystems). The results of DNA sequence analysis were compared with the published reference mtDNA sequence (GenBank, accession number J01415) using Autoassembler® software (Applied Biosystems). A 400 bp fragment of the D-Loop (nucleotide position: 190–590) containing the D310 homopolymeric C-tract from each patient was analysed. Any mtDNA sequences that differed between tumour and matched lymphocytes mtDNA were scored as somatic mutations. All somatic mutations found were further validated by a new independent amplification and sequencing.

Statistical analysis

The x²-test was used to determine the relationship between each categorical variable and D-Loop mutations, and a t-test to determine the relationship between quantitative variables and D-Loop mutations. Survival curves were constructed using the Kaplan–Meier method and compared using the log-rank test. The
median time of survival was used to summarise the survival data. These statistical tests were performed using the STATA software (STATA 7.0; College Station, TX, USA). A P-value < 0.05 was used to indicate statistical significance.

RESULTS
Displacement-Loop mutations
Displacement-Loop sequence analysis was performed in 109 patients. A total of 25 somatic D-Loop mutations were identified in 23 of the 109 (21%) tumours (Table 2). The majority of the mutations were located in the D310 mononucleotide repeat (19 out of 25, 76%). These mutations were insertions or deletions of one (n = 15) to several (n = 4) base pairs. Six mutations were found outside the D310 sequence. Among them, five were substitution of one base pair. The last one was a CA deletion at the nucleotide position 514. Two patients (nos. 22 and 153) had two mutations: in each of them, a D310 mutation coexisted with a D-Loop mutation located outside the D310 repeat. Among all mutations, 10 (40%) were homoplasmic and 15 (60%) were heteroplasmic. Homoplasmic mutations were significantly more frequent in the group of D-Loop mutations located outside the D310 repeat (5/6) than in the group of D310 mutations (five out of 19) (83 vs 26%, P = 0.02).

The D310 sequence is polymorphic in the human population. The number of cytosines in the 7-bp tract varied from 6 to 13 and the most frequent sequences for the D310 region are C7TC6, C8TC6 and C9TC6. In our series, the D310 sequence in nonmalignant tumour tissue. HNSCC = head and neck squamous cell carcinoma; D-Loop = Displacement-Loop.

Table 2 Summary of D-Loop somatic mutations found in the 109 HNSCC patients

| Patients number | Nucleotide position | DNA (N→T)* | mtDNA mutation status |
|-----------------|---------------------|------------|----------------------|
| 16              | 303–309             | C7→C7/C9   | Heteroplasmy          |
| 22              | 214                 | A→G        | Homoplasmy            |
| 23              | 514                 | (CA)5→(CA)4| Homoplasmy            |
| 28              | 314                 | C→T        | Homoplasmy            |
| 32              | 303–309             | C8→C7      | Homoplasmy            |
| 42              | 303–309             | C7→C7/C9   | Heteroplasmy          |
| 48              | 303–309             | C8/C9→C8/C10| Heteroplasmy       |
| 53              | 303–309             | C8→C8/C9   | Heteroplasmy          |
| 63              | 303–309             | C8/C9→C8   | Heteroplasmy          |
| 82              | 303–309             | C7→C7/C7   | Homoplasmy            |
| 110             | 303–309             | C9/C10→C9  | Homoplasmy            |
| 114             | 303–309             | C8/C9→C9/C10| Heteroplasmy       |
| 118             | 213                 | A→G        | Homoplasmy            |
| 127             | 303–309             | C8→C8/C9   | Heteroplasmy          |
| 141             | 303–309             | C9→C8      | Homoplasmy            |
| 146             | 408                 | T→A        | Homoplasmy            |
| 153             | 229                 | C→T        | Homoplasmy            |
| 155             | 303–309             | C7→C9/C10  | Heteroplasmy          |
| 161             | 303–309             | C8→C7/C8   | Heteroplasmy          |
| 167             | 303–309             | C8/C9→C8/C9| Heteroplasmy          |
| 180             | 303–309             | C9→C8/C9   | Heteroplasmy          |

* N = normal tissue; T = tumour tissue. HNSCC = head and neck squamous cell carcinoma; D-Loop = Displacement-Loop.

The analysis of clinicopathologic variables showed a significant difference in tumour site between tumours with and without D-Loop mutation (P = 0.03). Indeed, among D-Loop-mutated tumours, 47.8% were located in the hypopharynx as compared to 18.6% in the group of nonmutated tumours (Table 1). Moreover, presence of D-Loop mutation was significantly associated with tobacco consumption: the mean number of pack-year in the group of patients with tumour D-Loop mutation was 48 ± 3 compared to 37 ± 2 for patients without mutation (P = 0.01). The analysis of other clinicopathologic variables showed no correlation between the presence of a tumour D-Loop mutation and, respectively, age, gender, tumour stage and TP53 mutation (Table 1).

Association of D-Loop mutations and response to chemotherapy and survival
Tumours were classified according to the decrease in tumour size after 5FU – cisplatin-based chemotherapy as it was defined in the Materials and Methods section. In all, 72 patients (66.1%) were

Table 3 Summary of the germline polymorphisms found in the 109 head and neck squamous cell carcinoma patients

| Patients number | Nucleotide substitution | Nucleotide position | Reported polymorphism (databasea) |
|-----------------|-------------------------|---------------------|-----------------------------------|
| 16              | G→A                    | 200                 | Yes                               |
| 20              | G→A                    | 204                 | Yes                               |
| 48              | 206                     | G→A                 | Yes                               |
| 27              | 32, 52, 128, 150        | G→A                 | Yes                               |
| 82              | T→C                    | 239                 | Yes                               |
| 199             | A→G                    | 240                 | Novel                            |
| 41              | 54, 171                 | C→T                 | 242                               |
| 133             | A→G                    | 249                 | Novel                            |
| 48              | T→C                    | 250                 | Yes                               |
| 44              | A→G                    | 257                 | Yes                               |
| 171             | C→T                    | 295                 | Yes                               |
| 9, 13, 18, 26, 31, 35, 65, 72, 76, 84, 87, 88, 116, 164, 169, 177, 183, 190 | C7→C8 | 303–309 | Yes |
| 20              | 133, 156, 159           | C7→C9               | 303–309                           |
| 22              | C→T                    | 456                 | Yes                               |
| 28              | 32, 41, 52, 54, 128, 171| C→T                 | 462                               |
| 44              | 119                     | T→C                 | 477                               |
| 28              | 32, 41, 52, 54, 128, 171| T→C                 | 489                               |
| 26              | 83, 194, 208            | C→T                 | 497                               |
| 47              | A→C                    | 512                 | Yes                               |
| 23              | 32, 71, 72, 83, 130, 146, 172, 195 | (CA)5→(CA)4       | 514                               |
| 192             | (CA)4                   | 514                 | Yes                               |
| 9, 22           | (CA)5→(CA)7             | 514                 | Yes                               |
| 65              | A→G                    | 533                 | Yes                               |
| 165             | C→T                    | 534                 | Novel                            |

*MITOMAP database available on web: www.mitomap.org
responders and 37 (33.9%) were nonresponders. No correlation was found between the presence of tumour D-Loop mutation and response to neoadjuvant chemotherapy (Table 1).

The presence of D-Loop mutation was not a prognostic factor: the 5-year overall survival of patients with tumour D-Loop mutation was 81% compared to 70% for patients without mutation ($P = 0.71$, Figure 1).

**DISCUSSION**

In the past few years, somatic mtDNA mutations have been identified in several types of human tumours (Polyak et al, 1998; Fliss et al, 2000; Maximo et al, 2000; Richard et al, 2000; Yeh et al, 2000; Hibi et al, 2001b; Kirches et al, 2001; Liu et al, 2001; Sanchez-Cesperdes et al, 2001; Nomoto et al, 2002; Lievre et al, 2005), including HNSCC (Fliss et al, 2000; Sanchez-Cesperdes et al, 2001; Ha et al, 2002; Poetsch et al, 2004). In the present study, which is the largest series of HNSCC analysed for mtDNA mutations in the literature, we report a 21% frequency of D-Loop somatic mutations. This frequency is similar to that obtained by Fliss et al (2000) who performed a sequence analysis of 80% of the mitochondrial genome and found a D-Loop mutation in three of 13 (23%) HNSCC patients. However, other studies on small series of patients reported more frequent D-Loop mutations, ranging from 37 to 61% (Sanchez-Cesperdes et al, 2001; Hibi et al, 2002; Poetsch et al, 2004) of the cases. In these studies, D310 repeat emerged as a mutational hotspot. On 67 primary HNSCC from 56 patients, Poetsch et al (2004) sequenced two parts of the D-Loop and two mitochondrial genes (MTND1 and MTND5). They found all mtDNA mutations in a part of the D-Loop and a mtDNA microsatellite instability, defined as insertions or deletions in the D310 repeat, in 42% of the tumours. Another study showed 66% of D-Loop mutations in 18 oral cancers of betel quid chewers (Tan et al, 2003), which however cannot be really assimilated to HNSCC of tobacco and alcohol consumers. The vast majority of these mutations were located between nucleotides 204 and 489 and 44% of the tumours harbour mutations in the D310 repeat. These results led us to focus our sequence analysis on a 400 bp fragment of the D-Loop (nucleotide position: 190–490) containing the D310 C-tract which was found to be a hotspot of mutations since more than 80% of the D-Loop mutations were located in this sequence, as it was suggested previously (Sanchez-Cesperdes et al, 2001; Ha et al, 2002). The differences in D-Loop mutation frequency observed between our study, that of Fliss et al and the other studies remain to be explained. Overestimation of gene mutation rates is frequently observed in series of small sample size, which is the case of previous studies that have specifically addressed the D-Loop mutation frequency issue in HNSCC. As a consequence, our study gives new information as regards to the frequency of D-Loop mutations in HNSCC that has likely been overestimated in previous studies of small samples size. Another explanation may be differences in distribution of tumour sites between studies. We showed that tumours located in the hypopharynx (24.8% of all tumours) were significantly more mutated than tumours in other sites. Proportion of hypopharynx tumours is not known in the other studies (Fliss et al, 2000; Sanchez-Cesperdes et al, 2001; Ha et al, 2002; Poetsch et al, 2004). So, we can speculate that a higher proportion of tumours located in the hypopharynx would be associated with a higher frequency of mtDNA mutation. Moreover, in the present study, the prevalence of tumour D310 mutations increased significantly with the number of cytosines in the D310 sequence of matched normal tissues. According to these findings, the frequency of D-Loop mutations may depend on the proportion of the different D310 sequences in the normal tissues ($C_{7-9}$ to $C_{19}$), which is probably not the same in the different studies. Finally, the mtDNA mutagenesis process in oral squamous cell carcinoma of betel quid chewers is likely to be different to that of cigarette smokers. Betel quid contains tender areca nuts and lime that have been shown to generate ROS and induce oxidative DNA damage (Nair et al, 1987; Stich and Anders, 1989), which can initiate or promote oral carcinogenesis. These betel quid compounds could therefore preferentially target mtDNA, which may explain the high proportion of oral tumours with mtDNA mutation observed in betel quid chewers (Tan et al, 2003), compared to that observed in cigarette smokers.

With a frequency of more than 20% in HNSCC, D-Loop mutations may be an interesting molecular marker in the evaluation of the tumorigenic potential of head and neck lesions in individuals at high risk of this cancer. They were shown to be an early event in head and neck carcinogenesis (Ha et al, 2002). Their frequency was 22% in the earliest head and neck premalignant lesions and increased with the degree of dysplasia to reach 50% in lesions of severe dysplasia and 61% in carcinomas in situ in a recent study (Ha et al, 2002). Similar results were obtained in prostate (Jeronimo et al, 2001) and oesophagus adenocarcinoma (Miyazono et al, 2002) where identical D-Loop mutations were found both in primary tumours and corresponding premalignant lesions, which is consistent with a process of clonal evolution. These data suggest that D-Loop mutations could be considered as a cancer biomarker that may be useful for the early detection of HNSCC. It is all the more important since mtDNA mutations were reported to be easily detectable in bodily fluids and serum of cancer patients (Fliss et al, 2000; Hibi et al, 2001a; Jeronimo et al, 2001; Parrella et al, 2001; Nomoto et al, 2002). As a consequence, it would be interesting to search for D-Loop mutations in saliva and serum of a large series of HNSCC patients in order to evaluate their relevance in association with other tumour-specific molecular alterations in the screening of this cancer in alcohol and tobacco consumers.

We analysed for the first time the correlation between mtDNA mutations and clinicopathologic characteristics in a series of HNSCC patients. We found that tobacco consumption was significantly more important in the group of patients with tumour D-Loop mutation than in those without mutation. It is not very surprising since cigarette smoke contains several mutagenic ROS-forming substances and carcinogens that can cause DNA damages, especially in mtDNA that has been proved to be more susceptible to oxidative damages than nDNA (Marcelino and Thilly, 1999). In the same way, mtDNA content alterations have been recently shown to be associated with smoking (Ballinger et al, 1996; Lee et al, 1998). Our results also show an association between tumour site and D-Loop mutation, demonstrating that hypopharyngeal tumours were significantly more frequent in the group of mutated tumours compared to that of nonmutated tumours. This association remains unclear. One explanation could be a difference in exposition degree to mtDNA mutagenic agents between hypopharynx and other tumour sites. It was shown a
significant higher risk for cancer of the hypopharynx than for larynx cancer with alcohol drinking, which may be explained by the fact that hypopharynx enters in contact with the bolus (alcohol) and the air (tobacco smoke) while air pass through larynx but not the bolus (Esteve et al, 1996; Menvielle et al, 2004). Alcohol is known to cause oxidative stress through production of ROS (Hoek et al, 2002) and ethanol consumption has been reported to induce oxidative damage to mtDNA with increased levels of 8-hydroxydeoxyguanosin in rats (Cahill et al, 1997). The abasic sites and oxidised bases generated could so be responsible for mtDNA mutations. We did not found any correlation between D-Loop mutation and gender. This may be explained by the disequilibrium between the group of male (n = 98) and female (n = 111) subjects, which is the standard regimen in this case, has shown objective response rates of 71%, with complete response rates of 18% (Altundag et al, 2005). This neoadjuvant chemotherapy do not improve survival but is of interest in terms of organ preservation strategies in responder patients (Laccourreye et al, 1999) and has been shown to be curative in complete clinical responders cases with a cancer of the pharyngolarynx (Laccourreye et al, 2001). Therefore, identification of factors linked to response to neoadjuvant chemotherapy in HNSCC may help in the selection of patients who could benefit from an organ preservation. Some studies have revealed that the drug-resistance phenotype can be significantly associated with resistance to apoptosis induced by drugs (Landowski et al, 1997). Mitochondria is known to play a pivotal role in apoptosis (Green and Kroemer, 2004), and it was suggested that mtDNA determines the cellular response to some cancer therapeutic agents through a mechanism that could be an apoptosis modulation (Singh et al, 1999; Hail et al, 2001). We recently found that D-Loop mutations were associated with poor prognosis and absence of benefit from adjuvant 5FU chemotherapy in colon cancers (Lievre et al, 2005). Given these results, as all patients in our study received a neoadjuvant chemotherapy with 5FU – cisplatin, we investigated the association between D-Loop mutations and response to this treatment. No association with prognosis or with response to 5FU-cisplatin was found. These results suggest that mtDNA do not play the same role in the cytotoxicity of these two anticancer drugs. As 5FU interferes with the apoptotic process (Sakaguchi et al, 1994), we hypothesised in our previous study (Lievre et al, 2005) that the potential respiratory chain alteration induced by D-Loop mutations could explain in part the absence of benefit from 5FU we observed. On the contrary, Liang et al demonstrated that mtDNA depletion increased the sensitivity of cisplatin – induced apoptosis in U937 cells when compared to parental controls containing mtDNA (Liang and Ullyatt, 1998). According to these findings, we can speculate that D-Loop mutations may lead both to resistance to 5FU and increased sensitivity to cisplatin, which may explain that no link was observed between these mutations and response to the combined therapy in the present study. However, the power of our study remains low as regards to the small sample size and the unequal distribution of patients into the two groups considered for the analysis (23 tumours with D-Loop mutations, 86 tumours without), and larger studies are needed to confirm this result.

In conclusion, our data suggest that D-Loop mutations should be considered as a cancer biomarker that may be useful for the early detection of HNSCC in individuals at risk of this cancer. The presence of these mutations in saliva and serum of tobacco and alcohol consumers should be investigated in further studies in order to evaluate their relevance in the screening of these cancers in association with other tumour-specific molecular alterations.

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