Clonality test of multiple lung cancers by mitochondria DNA analysis

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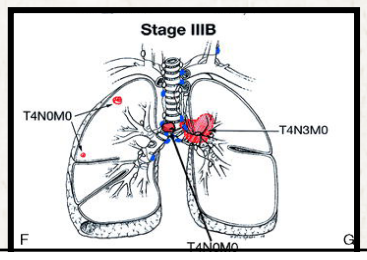
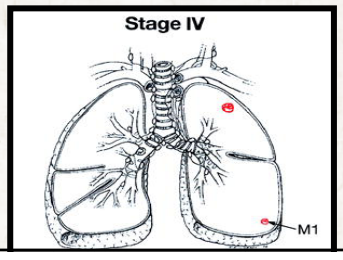
Multiple lung cancer

- 1 - 7% (either synchronous or metachronous)
- Multiple primary lung cancer
  - Each should be staged separately
  - Highest stage should be recorded, with separate coding to identify multiple primary tumors (pTm)
- Intrapulmonary metastasis
  - Separate tumor nodule (s) in same lobe (T4)
  - Distant metastasis present, or separate tumor nodule (s) in a different lobe (ipsilateral or contralateral) (M1)





Criteria for Diagnosis of Multiple Primary Lung Carcinomas

By Matini and Melamed, J. Thorac. Cardiovasc Surg, 1975: 70:606

Synchronous primary tumors

1. Tumors physically distinct and separate
2. Histology

a. different

b. same, but in different location, if

(1) origin from carcinoma in situ

(2) no lymphovascular invasion to both tumors

(3) no extrapulmonary metastases at time of diagnosis

Metachronous primary tumors

1. Histologically different
2. Similar histology, but

a. free interval between tumors of ≥ 2 years

b. origin from carcinoma in situ

c. metachronous second primary tumor with

(1) no LV tumor invasion to both tumors

(2) no extrapulmonary metastases at time of Dx.

Intrapulmonary metastasis

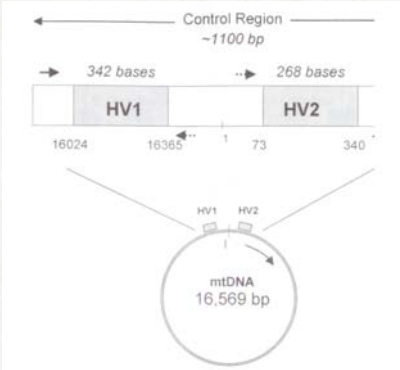
- Located in at least different lobar segments
- Tumors with same histology
- Demonstrating carcinoma in LV common to both tumors
- Lacking in situ component
- Occurring in setting of extrapulmonary metastases

Clonality Tests

- HUMARA assay in females using X-chromosome inactivation
  - Different inactivation pattern in indivisual cells
- P53 mutation analysis
  - Most frequent genetic alteration of lung ca
  - Widely distributed, involving various codons in exon 5-8
  - Occur early, and preserve during progression
- K-ras, EGFR
- LOH on 9p, 3p, 1p (early chromosomal changes)
- Microsatellite instability

Mitochondria DNA

- Small circular genome (16569 base pairs)
- Hundreds to thousands of mitochondria in each cells
- 2-10 copies in each mitochondria
- Coding region
  - 37 genes for coding for products in the oxidative phosphorylation process
  - 22 tRNA, 2 rRNA, 13 genes
- Control region (D-loop)
  - Variation between indivisuals
  - Hypervariable region 1 (HV1)
  - Hypervariable region 2 (HV2)
  - Hypervariable region 3 (HV3)
    - 137 bp (438-574)



Characteristics	Nuclear DNA	mt DNA
Size of genome	3 billion bp	16,569 bp
Copies per cell	2	Can be >1000
Structure	Linear; packages in chromosomes	Circular
Inherited from	Father and mother	Mother
Generational recombination	Yes	No
Unique	Unique to indivisual	Not unique to indivisual
Mutation rate	Low	At least 5-10 times nucDNA

Ref) Jonh M. Butler Forensic DNA typing 1st edition p122

Heteroplasmy

- Condition whereby more than one mtDNA type exist within an indivisual
- Length heteroplasmy
  - Multiple populations of mtDNA containing polycytosine stretches (C-stretches) or CA dinucleotide repeats of various lengths
  - General and less population specificity
  - C-stretches
    - 16184 - 16193 in HV1 (36% in Korean)
    - 303 - 315 in HV2 : (69% in Korean)
    - 568 - 573 in HV3
    - DM, dilated cardiomyopathy (DCM), tumor
  - CA repeats: 514 – 523 in HV3 (0.6% in Korean)
- Point heteroplasmy
  - Presence of two different populations of mtDNA varying from each other at a given nucleotide position (2.4% in Korean)

Mitochondrion. 2007 Sep;7(5):347-53

Relationship between mitochondrial DNA mutations and clinical characteristics in human lung cancer

Jin X et al.

- Material and methods
  - 55 lung cancer patients
  - Lung cancer tissue, normal lung tissue, peripheral blood
  - Complete mt DNA sequencing
- Results
  - 56 mutation in 33 out of 55 cases
    - 48 point mutation, 4 single nucleotide insertion, 4 single nucleotide deletion
    - 18 mutation in D-loop
    - Absent in same locus mutation between cases
  - 388 polymorphism
    - 77 polymorphism in coding region
  - No association with age, gender, smoking history, histologic type, and stage

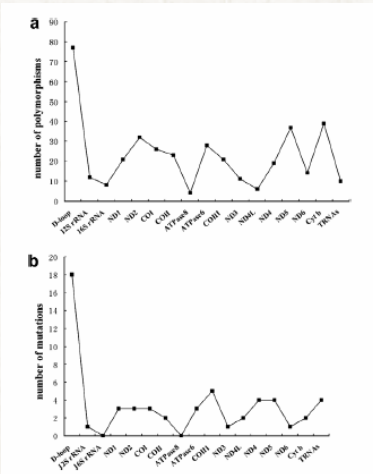


Fig. 1. The distribution of mtDNA polymorphisms (a) is similar to that of mutations (b) in mitochondrial genome. The ordinate shows the number of polymorphisms (a) and mutations (b). All of 22 tRNA-coding sequences were combined as a 'gene'.

Cancer Res. 2001 Oct 1;61(19):7015-9.

Identification of a mononucleotide repeat as a major target for mitochondrial DNA alterations in human tumors

Sanchez-Cespedes M et al.

- 247 cancer patients, including 100 lung ca
- Tumor tissue, normal tissue from lymphocytes
- C-stretches in HV2

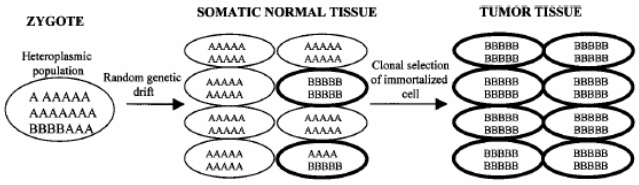


Fig. 3. Proposed model for the generation of neutral D310 homoplasmic alterations in tumor mtDNA. Unequal partitioning of genomes during cytokinesis can lead to differences between daughter cells in somatic tissue through stochastic segregation and random genetic drift. *A*, *A/B*, and *B* represent the mitochondria organelles harboring the predominant mitochondrial genomes *A*, *B*, or heteroplasmic (both A and B). After clonal selection of an immortalized cell attributable to nuclear oncogene mutations, tumor tissue harbors the homoplasmic B variant. Tumors may also achieve homoplasmicity from heteroplasmic A/B cells by recapitulating this process in conjunction with clonal expansion driven by additional nuclear mutations. Differences in observed frequencies of neutral polymorphism variants in various tumors types may be attributable to the number of clonal expansions preceding clinical presentation (30).

Purpose

- Clonality test of multiple lung cancer using tool for human identification
- Weak DNA repair function of mitochondria DNA

- Point mutation
- Length homoplasmy from length heteroplasmy
  => clonal selection & expansion
- Aquired point or length heteroplasmy

Material and methods (1)

- 16 lobectomy or peumonectomy samples
  from 2004 to 2007 in severance hospital
- Clinicopathologic analysis
  - Age and sex
  - Location
  - Size
  - Histologic type
  - Lymphovascular invasion
  - Carcinoma in situ
  - Lymph node or extrapulmonary metastasis

Material and methods (2)

- DNA extraction
  - Paraffine block selection
    - Normal, tumor 1, tumor 2
  - QIAamp® DNA Mini Kit
- Autosomal STRs genotyping
  - PCR (Idfiler, minifiler)=> Genotyping by using capillary electrophoresis
- Mitochondria DNA analysis
  - Control region sequence
    - PCR => Squencing by using capillary electrophoresis
  - Length heteroplasmy
    - C-stretches in HV2
    - CA dinucleotide repeats in HV3
      - PCR => Genotyping by using capillary electrophoresis

Results (1)

Table 1 Summery of clinical and histologic findings

Patient No	Age	Sex	Presentaton	No. of lesions	Histolgy of tumor 1	Histology of tumor 2	Distribution	LN mets	MM criteria
1	60	M	syn	2	SQ	SQ	different lobe	no	?
2	74	M	syn	2	SQ	SQ	different lobe	yes	I
3	54	M	syn	3	SQ	SQ	same lobe	no	I
4	54	F	meta	2	AD	AD	different lobe	no	?
5	68	M	syn	2	SQ	SQ	same lobe	no	I
6	63	F	syn	2	AD	AD	different lobe	no	D
7	49	M	syn	2	SQ	SQ	same lobe	yes	I
8	75	F	syn	5	AD	AD	different lobe	yes	?
9	47	F	syn	2	AD	BAC	different lobe	no	D
10	70	M	syn	2	SQ	AD	different lobe	no	D
11	61	M	syn	2	GC	GC	different lobe	yes	I
12	68	M	syn	3	SQ	SQ	same lobe	yes	I
13	55	M	syn	2	AD	AD	same lobe	no	?
14	63	M	syn	2	AD	AD	same lobe	yes	?
15	78	M	syn	2	SQ	SQ	different lobe	no	?
16	68	F	syn	2	AD	AD	same lobe	no	D

Results (2)

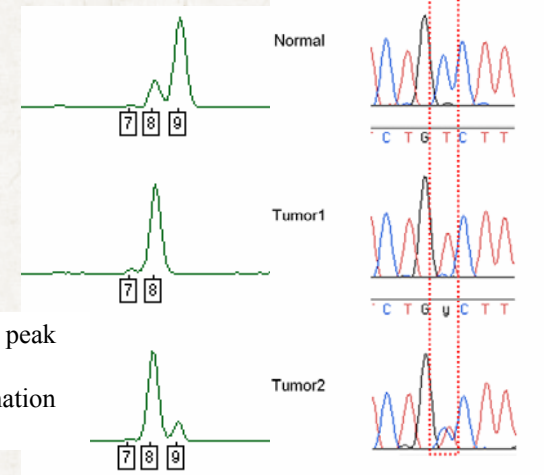
- Microsatellite instability: 4 cases
- LOH: 3 cases (2 cases: same loci in both tumors, 1 case in one tumor)
- Length heteroplasmy
  - C-stretches in HV2: 11 cases
    - Increased polymorphism: 2 cases
    - Decreased polymorphism: 2 cases
    - Major peak change: 4 cases
  - C-stretches in HV3: 1 case (Homoplasmy => heteroplasmy)
  - CA dinucleotide repeats: 1 case (Homoplasmy => heteroplasmy)
- Point heteroplasmy or point mutation: 8 cases

Sample No	STR (MSI)	LOH	C-stretches	CA repeats	PH	
LC01-N			7	5	16092C	
LC01-T1	5 out of 13		7	5	16092M(A+C)	
LC01-T2	4 out of 15		7	5	16092M(A+C)	
LC02-N			7	5	189A	
LC02-T1	1 out of 15		7	5	189A	
LC02-T2	0 out of 15	+	7	5	189G	
LC03-N			7,8,**9**	4	131C	
LC03-T1	0 out of 15		7,**8**	4	131T	
LC03-T2	0 out of 15		7,**8**,9	4	131Y(C+T)	
LC04-N			8,**9**	5		
LC04-T1	0 out of 15		**8**,9	5		
LC04-T2	0 out of 15		8,**9**	5		
LC05-N			7	5	16189T	
LC05-T1	0 out of 15	+	7	5	16189Y(C+T)	
LC05-T2	0 out of 15	+	7	5	16189Y(C+T)	
LC06-N			7,**8**,9	5		
LC06-T1	0 out of 15		7,8,**9**	5		
LC06-T2	0 out of 15		7,**8**,9	5		
LC07-N			7,**8**	4		
LC07-T1	0 out of 15		7,**8**	4		
LC07-T2	0 out of 14		7,**8**	4		
LC08-N			7	5	16093C	C6 in HV3
LC08-T1	0 out of 15		7	5	16093C	C6 in HV3
LC08-T2	0 out of 15		7	5	16093T	C**6**,7 in HV3

Sample No	STR (MSI)	LOH	C-stretches	CA repeats	PH	
LC09-N			7,**8**	4	16390G	
LC09-T1	0 out of 15		7,**8**	4	16390R(G+A)	
LC09-T2	0 out of 15		7,**8**	4	16390G	
LC10-N			7,**8**	5	204T	
LC10-T1	0 out of 15		7,**8**	5	204C	
LC10-T2	0 out of 15		7,**8**,9	5	204T	
LC11-N			7,8	5		
LC11-T1	1 out of 15	+	7,8	5		
LC11-T2	1 out of 15	+	7,8	5		
LC12-N			7,**8**,9	5		
LC12-T1	6 out of 15		**4**,8,9	5		
LC12-T2	0 out of 14		**4**	5		
LC13-N			7,**8**	5		
LC13-T1	0 out of 15		7,**8**	5		
LC13-T2	0 out of 15		7,**8**	5		
LC14-N			7,**8**,9	5		
LC14-T1	0 out of 15		7,**8**,9	5		
LC14-T2	0 out of 15		7,**8**,9	5		
LC15-N			7,**8**,9	5	16291C	183A
LC15-T1	0 out of 15		7,**8**,9,10	5	16291Y(C+T)	183R(A+G)
LC15-T2	0 out of 15		7,**8**,9,10	5	16291Y(C+T)	183R(A+G)
LC16-N			7	5		
LC16-T1	0 out of 15		7	5		
Lc16-T2	0 out of 15		7	4,5		

Case 3

- Intrapulmonary metastasis by **Matini and Melamed**
- Microsetellite instability (Autosomal STRs)
  - Tumor 1 : absent
  - Tumor 2 : absent
- C-stretches:
  - Normal: 7, 8, 9
  - Tumor 1: 7, 8
  - Tumor 2: 7, 8, 9
- CA repeats:
  - Normal: 4
  - Tumor 1: 4
  - Tumor 2: 4

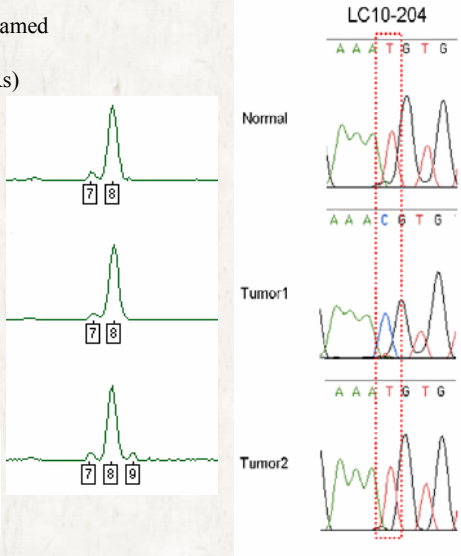


1. Decreased polymorphism & major peak change in length heteroplasmy
2. Point mutation & normal contamination

=> Intrapulmonary metastasis

Case 10

- Double primary tumor by Matini and Melamed
- Microsetellite instability (Autosomal STRs)
  - Tumor 1 : absent
  - Tumor 2 : absent
- C-stretches:
  - Normal: 7, 8
  - Tumor 1: 7, 8
  - Tumor 2: 7, 8, 9
- CA repeats:
  - Normal: 5
  - Tumor 1: 5
  - Tumor 2: 5



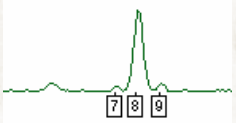
1. Point mutation in tumor 1
2. Aquired length heteroplasmy in tumor 2

=> Double primary tumor

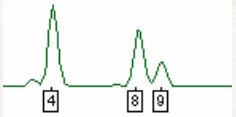
Case 12

- Intrapulmonary metastasis by **Matini and Melamed**
- Microsetellite instability (Autosomal STRs)
  - Tumor 1 : present in 6 out of 15
  - Tumor 2 : Absent
- C-stretches:
  - Normal: 7, 8, 9
  - Tumor 1: 4, 8, 9
  - Tumor 2: 4
- CA repeats:
  - Normal: 5
  - Tumor 1: 5
  - Tumor 2: 5

LC12-Normal



LC12-Turmor 1



LC12-Turmor 2

1. Deletion of C-stretches
2. Homoplasmy from length heteroplasmy
3. Different MSI pattern

=> Intrapulmonary metastasis

Patient No	MM criteria	Clonality study
1	?	I
2	I	? (favor D)
3	I	I
4	?	?
5	I	I
6	D	?
7	I	?
8	?	? (favor D)
9	D	D
10	D	D
11	I	?
12	I	I
13	?	?
14	?	?
15	?	I
16	D	?

Discussion

- Limitation
  - Point heteroplasmy vs. point mutation & normal contamination
  - Discrimination power of point mutation
  - The meaning of major peak change of length heteroplasmy
    - Clonal selection (?)
  - The meaning of new length heteroplasmy
  - The proven clonality test (p53 et al.) do not performed.
    - Limitation in evaluation of usefulness as clonality test tool
- Mitochondria DNA analysis can be used as at least assistant tool.