Mapping of homozygous deletions in chromosome 3p regions affected in major epithelial tumors using real-time PCR

V.Senchenko[1,2,3], E.Braga[4], J.Liu[2], V.Loginov[4], V.Kashuba[2,5], R.Garkavtseva[6], N. Mazurenko[6] , F.Kisseljov[6], L.Kisselev[1], E.Zabarovsky[2]

[1]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia; [2]Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Sweden; [3]Center "Bioengineering", Russian Academy of Sciences, Moscow, Russia; [4]Russian State Genetics Center, Moscow, Russia; [5]Institute of Molecular Biology and Genetics, Ukrainian Academy of Sciences, Kiev, Ukraine; [6] Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russia

Abstract

We searched for chromosome 3p homo- and hemizygous losses in 23 lung cancer cell lines, 53 renal cell and 22 breast carcinoma biopsies using 31 microsatellite markers located in frequently deleted 3p regions. In addition, two STS markers (NLJ-003 and NL3-001) located in the Alu-PCR clone 20 region (AP20) and lung cancer) region (LUCA, respectively, were used for real-time PCR. We found frequent (10-18%) homozygous deletions (HDs) in both 3p21.3 regions in the biopsies and cell lines. In addition, we discovered that amplification of 3p is a very common (15-42.5%) event in these cancers and probably in other epithelial malignancies.

Real-time PCR should that aberrations of either NLJ3-001 or NL3-001 were detected in more then 90% of all studied cases. HDs were frequently detected simultaneously both in NLJ3-001 or NL3-001 loci in the same tumor. This fact suggests that TSGs in this regions could have a synergistic effect .

The exceptionally high frequency of chromosome aberrations in NLJ3-001 and NL3-001 loci suggests that multiple TSGs involved in different malignancies are located very near this markers.

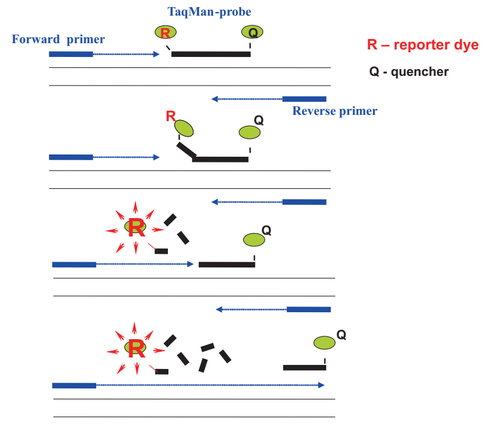
Precise mapping of 15 independent HDs in the LUCA allowed us to establish the smallest HD region in 3p21.3C located between D3S1568 (*CACNA2D2* gene) and D3S4604 (*SEMA3F* gene). This region contains 17 genes.

Mapping of 19 HDs in AP20 region resulted in the localization of the minimal region to interval flanked by D3S1298 and D3S3623 markers. Only 4 potential candidates have been discovered in this interval, namely *APRG1*, *ITGA9*, *RBSP3/HYA22* and *VILL* which need to be analysed.

Introduction

Localization, identification of oncogenes and tumor suppressor genes (TSGs) and their expression are fundamental for understanding the difference between normal and malignant tissues.

Real-Time PCR is the most effective method for the identification of genomic DNA deletions, duplications, amplifications and evaluation of gene expression level.



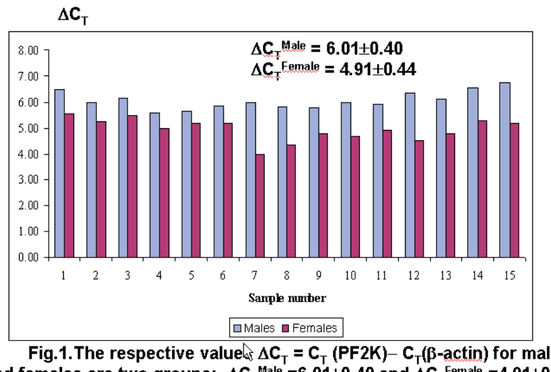
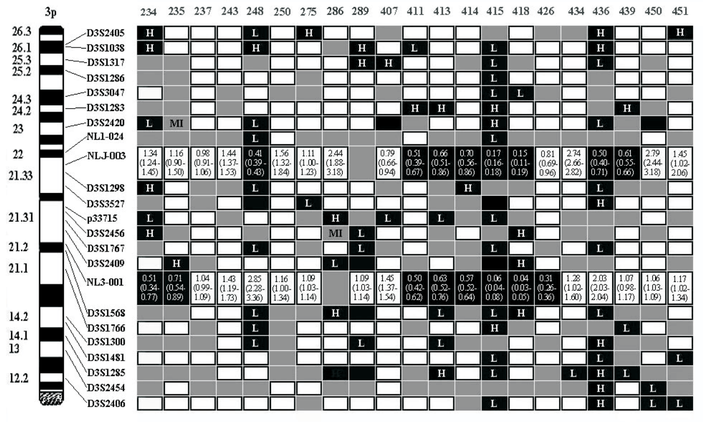


Fig.1.The respective values $\Delta C_T = C_T$ (PF2K) – C_T(β-actin) for males and females are two groups: ΔC_T^{Male} =6.01±0.40 and ΔC_T^{Female} =4.91±0.44. The amount of target (PF2K) for females, normalized to a reference (β-actin) and relative to target (PF2K) for males is: $N^{Female}/N^{Male}=2^{-\Delta\Delta CT}$=2.14(2.08-2.20).

Cervical cancer: real-time PCR and LOH for 3p deletion mapping

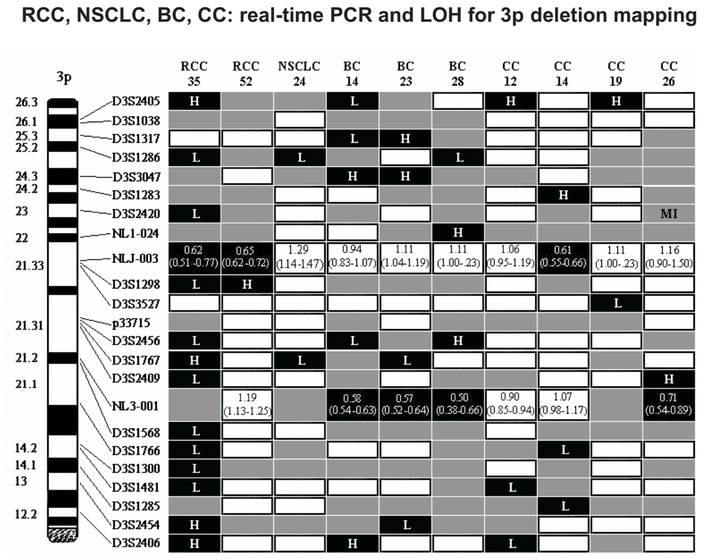


Comments: Black squares represent loss of heterozygosity and homozygous deletions; white squares, retention of heterozygosity and normal copy number; gray squares, multiplication and dashed boxes are non-informative cases. L and H inside black squares denote the type of allele lost.

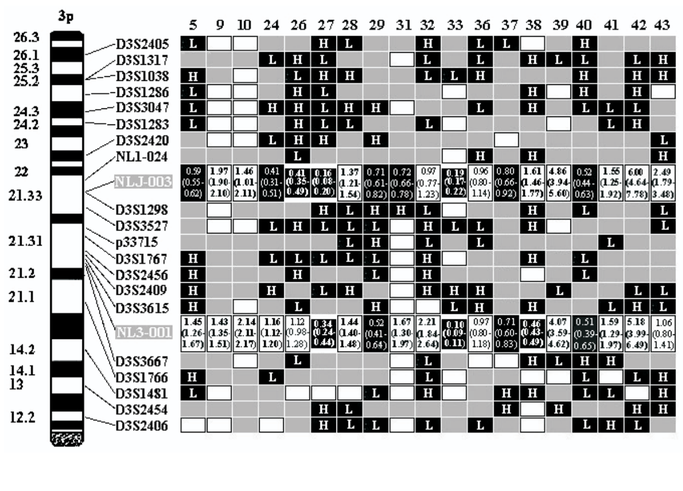
Comparison of real-time PCR and Southern blot analysis data

n/n	Clinical number	Relative DNA copy number NLJ-003 (3p21.3)	Relative DNA copy number NL3-001 (3p21.2-21.3)
1	234	1.36(1.13 – 1.63)	**0.51**(0.34-0.77)
2	235	1.16(0.90 – 1.50)	**0.71**(0.54-0.89)
3	237	0.98(0.91 – 1.06)	1.04(0.99-1.09)
4	243	1.24(1.17 – 1.31) **(1.12±0.21) Southern method**	1.43(1.19-1.73)
5	**244**	2.75(2.46 – 3.06)	1.65(1.53-1.78)
6	248	0.61(0.50-0.70)	2.85(2.48-3.29)
7	250	1.56(1.32 – 1.84)	1.16(1.00-1.34)
8	275	1.11(1.00 – 1.23)	1.09(0.83-1.43)
9	**284**	**0.36**(0.28 – 0.45)	**0.18**(0.10-0.33)
10	289	**0.78(0.60 – 0.90)** **(0.70±0.24) Southern method**	1.09(1.03-1.14)
11	290	0.80(0.57 – 1.10)	1.06(0.97-1.15)
12	**297**	2.39(2.19 – 2.62)	2.50(2.50-2.60)
13	341	1.19(0.96 – 1.46)	0.88(0.79-0.97)
14	407	**0.79(0.66 – 0.94)**	1.45(1.37-1.54)
15	**409**	**0.38**(0.30 – 0.48)	**0.49**(0.43-0.57)
16	411	**0.66**(0.51–0.71) **(0.87±0.34) Southern method**	**0.50**(0.42-0.62)
17	413	**0.61**(0.51 – 0.71)	**0.63**(0.52-0.76)
18	414	**0.70(0.56 – 0.86)**	**0.57**(0.52-0.64)
19	**415**	**0.17**(0.13 –0.23)	**0.06**(0.04-0.08)
20	**418**	**0.15**(0.11 – 0.[illegible])	**0.04**(0.03-0.05)
21	419	**0.78(0.54 – 0.85)**	1.51(1.19-1.93)
22	**426**	**0.44**(0.38 – 0.51) **(0.44±0.13) Southern method**	**0.31**(0.26-0.36)
23	427	1.37(1.25 – 1.51)	**0.62**(0.58-0.66)
24	**431**	2.74(2.66 – 2.82)	2.80(2.38-3.29)
25	434	0.98(0.94 –1.03) **(0.85±0.12) Southern method**	1.28(1.02-1.60)
26	436	**0.50**(0.40 – 0.71)	2.03(1.82-2.24)
27	**438**	**0.20**(0.19 – 0.22)	**0.10**(0.09-0.11)
28	439	**0.61**(0.55 – 0.66)	1.07(0.98-1.17)
29	440	0.88(0.87 – 0.90)	1.54(1.08-2.01)
30	448	1.08(0.98 – 1.14)	**0.60**(0.53-0.67)
31	450	2.79(2.44 – 3.18)	1.06(1.03-1.09)
32	**451**	**0.48**(0.39-0.58) **(0.44±0.14) Southern method**	1.17(1.01-1.34)
33	452	**0.57**(0.54-0.61) **(0.54±0.11) Southern method**	0.80(0.65-0.99)

RCC, NSCLC, BC, CC: real-time PCR and LOH for 3p deletion mapping



Renal Cell Carcinoma: real-time PCR and LOH for 3p deletion mapping



Lung cancer cell lines: real-time PCR data

Sample no. [a]	Histology	Cell line	Relative DNA copy number [b] NLJ-003 relative to ACTB and normalized to CBMI	NL3-001 relative to ACTB and normalized to CBMI
1	Adenosq.	H 125	0.64 (0.51-0.77)	0.55 (0.52-0.58)
2	SCLC	H 128	0.55 (0.47-0.63)	0.50 (0.46-0.55)
3	Large	H 460	***1.30 (1.10-1.50)***	0.52 (0.46-0.59)
4	SCLC	H 592	0.56 (0.50-0.62)	0.54 (0.46-0.62)
5	SCLC	H 719	0.88 (0.78-0.98)	1.05 (0.86-1.23)
6	SCLC	**H 740**	0.72 (0.60-0.83)	0.015 (0.010-0.019)
7	SCLC	H 865	1.13 (0.96-1.29)	1.01 (0.99-1.03)
8	SCLC	H 1045	0.84 (0.76-0.92)	nd
9	SCLC	H 1339	0.81 (0.64-0.98)	1.10 (0.96-1.24)
10	Adeno	H 1395	0.[illegible]0 (0.86-0.93)	0.51 (0.47-0.54)
11	SCLC	**H 1450**	0.78 (0.68-0.88)	0.009 (0.007-0.011)
12	SCLC	H 1514	0.44 (0.34-0.54)	nd
13	Adeno	H 1581	***1.37 (1.22-1.52)***	0.63 (0.61-0.65)
14	SCLC	H 1622	***2.50 (2.10-2.90)***	***2.90 (2.70-3.00)***
15	SCLC	H 1628	***1.37 (1.34-1.40)***	***1.70 (1.60-1.80)***
16	SCLC	**H 1672**	0.33 (0.25-0.42)	0.44 (0.38-0.51)
17	SCLC	**H 1688**	0.22 (0.17-0.28)	0.48 (0.34-0.62)
18	Adeno	**H 1693**	0.25 (0.18-0.32)	0.65 (0.57-0.73)
19	Squam.	H 1717	1.01 (0.93-1.08)	0.89 (0.85-0.92)
20	SCLC	H 1876	0.53 (0.40-0.67)	***1.80 (1.60-2.00)***
21	NSCLC	H 1993	0.84 (0.77-0.91)	nd
22	SCLC	H 2107	0.46 (0.39-0.53)	0.46 (0.41-0.51)
23	SCLC	H 2227	0.42 (0.34-0.51)	0.69 (0.50-0.95)

[a] Underlined bold samples contained homozygous deletions in one or both of the loci.
[b] Samples showed multiplication are in underlined bold italics and samples with white letters contained hemizygous deletions.

Table. Summary of the real-time PCR experiments

Cancer	NLJ-003 (cases / %) Amplification or multiplication.	Homozygous deletions	Hemizygous deletions	Total copy number changes	NL3-001 (cases / %) Amplification or multiplication	Homozygous deletions	Hemizygous deletions	Total copy number changes	Aberrations of either NLJ-003 or NL3-001 (cases / %)
RCC	18/34.0	8/15.1	18/34.0	44/83.0	17/42.5	4/10.0	8/20.0	28/72.5	49/92.5
BC	6/28.6	3/14.3	7/33.3	16/76.2	6/27.3	4/18.0	9/40.9	19/86.4	20/90.9
SCLC	4/17.4	3/13.0	14/60.9	21/91.3	3/15.0	2/10.0	12/60.0	17/85.0	22/95.7
CC	7/21.9	5/15.6	14/43.8	26/81.2	9/28.1	5/15.6	9/28.1	23/71.9	29/90.6

Table. Borders (shown black) of homozygous deletions near adjacent markers in 3p21.3T

n/n	Clinical sample	LOH analysis D3S1767 3p21.3	LOH analysis D3S2409 3p21.31	Real-time PCR NLJ-001 Relative to actin 3p 21.2-21.3	LOH analysis D3S1568 3p21.31	LOH analysis D3S1766 3p21.31
1	74 Rcc	LOH(L)	LOH(L)	0.34 (0.24-0.44)	Ni	Ni
2	193 RCC	LOH(L)	MI	0.52 (0.41- 0.64)	LOH(H)	Ni
3	197 RCC	Ret.	LOH(H)	0.10 (0.09 – 0.12)	LOH(L)	Ret.
4	315 BC	Ni	LOH	0.11 (0.10-0.12)	Ni	Ni
5	336 BC	Ret.	LOH	0.50 (0.38-0.66)	Ni	Ni
6	341 BC	Ret.	Ni	0.51 (0.44-0.58)	Ni	Ret.
7	345 BC	Ni	LOH	0.61 (0.56-0.66)	Ni	Ret.

Table. Borders (shown gray) of homozygous deletions near adjacent markers in 3p21.3C

Sample number	LOH analysis D3S2420 marker 3p21.3	LOH analysis NL1- 024 marker (3p22)	Real-Time PCR NLJ3-001 probe (3p 21.33)	LOH analysis D3S1298 marker (3p21.33)	LOH analysis D3S3527 marker (3p21.33)
10 (RCC)	NI	NI	0.59(0.55 – 0.62)	NI	NI
70 (RCC)	NI	NI	0.41(0.31 – 0.51)	NI	L
72 (RCC)	L	L	0.41(0.35 – 0.49)	NI	H
74 (RCC)	H	NI	0.16 (0.08 – 0.31)	H	ND
197 (RCC)	MI	R	0.19(0.17-0.22)	R	L
304 (RCC)	NI	NI	0.25(0.16 – 0.38)	L	ND
315(Breast)			0.19(015-0.24)		
317(Breast)			0.25(0.16 – 0.38)		R