P10-10 非小細胞肺癌におけるRAR-β, FHT, RASSF1A 遺伝子のメチル化の臨床病理学的意義

目的: ヒト肺がんにおいて第3番染色体短腕の欠失は高頻度
に認められており、RAR-β, FHT, RASSF1A 遺伝子はそれぞれ3p24, 3p14, 3p21.3より構成がん抑制遺伝子として同定されてきた。非小細胞肺癌においてこれらの遺伝子のメチル化の臨床病理学的意義を検討した。方法: 断片的に切除された非小細胞肺癌31例より全一部を抽出し
Methylation-Specific-PCR法を用いて解析した。結果: RAR-
β, FHT, RASSF1A 遺伝子のメチル化はそれぞれ31例
(26%), 43例 (36%), 35例 (29%)に認められた。それぞれ
の遺伝子のメチル化に相関は認められなかった。RAR-β 遺
伝子のメチル化は非喫煙者 (3/19, 10%)に比べ、喫煙者 (19/
61, 45%) P=0.037で高頻度に認められた。また、1期群例
(7/11, 17%)に比べ、IV期群群例 (19/47, 40%)に比べ、P=0.0057
で高頻度に認められた。FHT 遺伝子のメチル化はリンパ管
浸潤のない腫瘍(13/54, 24%)に比べ、リンパ管浸潤のある
腫瘍(28/59, 47%) P=0.0115で高頻度に認められ、血管浸
潤のない腫瘍 (20/68, 30%)に比べ、血管浸潤のある腫瘍
(28/59, 47%) P=0.0703で高頻度に認められた。また
RASSF1A 遺伝子のメチル化は扁平上皮がん (6/45, 13%)に
比べ、腺がん (28/72, 39%) P=0.0033で高頻度に認められ
た。結論: RAR-β, FHT, RASSF1A 遺伝子のメチル化に
よる不活化は非小細胞肺癌の発生と進展において様々な
役割を果たしていることが示唆された。

P10-11 Aberrant promoter methylation and histone
deacetylation of the COX-2 gene in Lung cancer

cell lines

Background: Immunostaining studies indicate frequent
up regulation of COX-2 expression in NSCLC that may be related
to increased invasiveness and lymph node metastases. By
contrast, expression in SCLC is weak. We studied the mecha
nism for the differential expression of COX-2 gene in lung
cancer cell lines. Methods: COX-2 mRNA expression assays
were examined by reverse transcription-PCR (RT-PCR) and
semi-quantitative real-time RT-PCR. Aberrant methylation of
the promoter region of COX-2 was studied by methylation
specific polymerase chain reaction (MSP) and bisulfite
DNA sequencing of cloned DNA of PCR amplions. The ef
fects of the demethylating agent 5-aza-2-deoxycytidine (5-
Aza) or a histone deacetylase inhibitor, trichostatin A (TSA)
were tested in expression negative cell lines. Results: COX-2
expression was present in all 18 NSCLC cell lines tested, but
was absent in 16/22 (73%) of SCLC lines. In the NSCLC lines
expression was 82 fold greater than in the 6 positive SCLC
lines. By MSP assay, methylation was absent in all of the
NSCLC lines. In SCLC lines three patterns were seen: Group
A, expression positive, methylation negative (n=5) Group B,
expression negative, MSP positive (n=6) and Group C, ex
pression positive, MSP negative (n=10). In addition, one cell
line had weak expression and weak MSP positivity. In methy
lation positive Group B cell lines, both 5-Aza and TSA restored
expression. However in methylation negative Group C cell
lines only treatment with TSA restored expression. Sequen
cing of the promoter region after bisulfite treatment confirmed
that cell lines of Groups A and C lacked methylation while
Group B cell lines were heavily methylated. Conclusions: 1)
COX-2 expression is down regulated in all SCLC cell lines ex
amined compared to NSCLC lines 2) expression was absent in
16 lines (73%) and 3) the mechanisms of lack of expression
were due to histone deacetylation, or by a combination of ab
errant methylation and histone deacetylation.