METHYLATION PROFILE OF IRF6 AND RARB GENE PROMOTERS IN NORMAL VULVAR TISSUES AND VULVAR CARCINOMAS

Rotondo J.C. (1), Bononi I. (1), Mazzoni E. (1), Torreggiani E. (1), Oton-Gonzalez L. (1), Iaquinta M.R. (1), Lanzillotti C. (1), Mazziotta C. (1), Tognon M. (1), Martini F. (1)

(1) Department of Morphology, Surgery and Experimental Medicine, School of Medicine, University of Ferrara, Ferrara (Italy)

Interferon regulatory factor 6 (IRF6) and retinoic acid receptor beta (RARB) play an important role in regulating cell proliferation and differentiation of the epithelia. IRF6 and RARB by modulating p63 and c-jun, respectively, arrest cell proliferation thus inducing differentiation. The promoter hypermethylation of tumor suppressor genes may favour the onset of cancers. In this study, IRF6 and RARB promoter methylation profiles were investigated in normal vulvar (NV, n=20) and pathological vulvar tissues from cancer-free lichen sclerosus (cfLS, n=20), cancer-associated lichen sclerosus (caLS, n=20), vulvar intraepithelial neoplasia (VIN, n=6, only IRF6) and vulvar cancer (VC, n=20) specimens. Methylation analyses were performed with the bisulphite-DNA treatment and PCR amplifications/sequencing of IRF6 and RARB promoters. IRF6 and RARB gene expressions, together with p63 and c-jun genes were analysed by qRT-PCR. IRF6 gene promoter was found to be hypermethylated in 10% cfLS, 20% VIN, 45% caLS and 80% VC ($p<0.01$, caLS and VC versus NV). IRF6 expression decreased 2.2-, 2.9-, 4.5- and 6.6-fold from cfLS, VIN, caLS to VC, respectively, whereas p63 was overexpressed in all specimens compared to NV ($p<0.05$). RARB gene promoter tested hypermethylated in 50% caLS, 55% cfLS and 90% VC ($p<0.01$, versus NV). Unlike IRF6, RARB was significantly down-expressed, 4.8-fold, only in VC ($p<0.01$, versus NV). Consistently, c-jun expression was 2.6-fold up-expressed in VC ($p<0.01$, versus NV). Interestingly, 2/18 (11.1%) VC, showing full methylation of RARβ gene promoter, were from females with tumor recurrences. IRF6 and RARB expressions are hampered by promoter hypermethylation in vulvar diseases and vulvar cancer. While IRF6 promoter hypermethylation occurs in a stepwise manner from vulvar LS to cancer, RARB promoter hypermethylation was found to be mainly associated to vulvar cancer. IRF6 and RARB dysregulations may play a role in caLS development and progression, respectively.