

Transactivation and expression patterns of Jun and Fos/AP-1 super-family proteins in human oral cancer

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Transcription factor activator protein-1 (AP-1) super-family is known to modulate expression of array of genes during development of many cancers and considered as an important target for modern therapeutics. But the role of AP-1 during development of human oral cancers is still poorly understood. Because oral cancer is one of the most common cancers in India and south-east Asia, we studied the activation and expression pattern of AP-1 family of proteins and mRNA in different stages of oral carcinogenesis. Gel-shift assay, western blotting, immunohistochemistry and northern blotting have been used to assess the binding activity and expression pattern of AP-1 family (c-Jun, JunB, JunD, c-Fos, FosB, Fra-1 and Fra-2) proteins and mRNA transcripts in a total of 100 fresh oral tissue specimens comprising precancer ($n = 40$), cancer ($n = 50$) and healthy control ($n = 10$). Constitutive activation of AP-1 with concomitant upregulated expression of majority of AP-1 family of proteins and mRNA was observed in cancer cases. Interestingly, almost all precancerous cases showed JunD homodimers, whereas c-Fos/JunD was the most prevalent complex found in cancer tissues. The overexpression of EGFR mRNA, p50:p50/NF- κ B homodimer formation, together with overexpression of pERK and c-Fos proteins in this study suggests an interesting cross talk between AP-1 and NF- κ B pathways in oral cancers. Thus, this study demonstrates differential expression and activation of AP-1 super-family proteins in relation to severity of lesion and their crucial role in human oral carcinogenesis.

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer and accounts for ~5% of all malignant tumors worldwide.¹ In India and South East Asia, it is the most common malignancy accounting to 50% of all malignant tumors. Most of the OSCC is attributed to smoking and alcohol consumption, whereas a proportion of oral cancers have been demonstrated to contain anogenital HPV infections.^{2,3}

The activator protein-1 (AP-1) super-family of transcription factor is a dimeric protein complex of structurally and functionally related members of Jun, Fos, ATF (activating transcription factors) and MAF (musculoaponeurotic sarcoma) protein families. The homodimerization of Jun proteins (c-Jun, JunB and JunD) or heterodimerization of Jun and Fos proteins (c-Fos, FosB, Fra-1 and Fra-2) generates a

transcriptionally active complex interacting through basic "leucine zipper" motif. AP-1 dimers regulate downstream target gene through interaction with DNA backbone of selective 8 base pair conserved sequence 5'TGAGCTCA 3' recognized as the TPA (12-*O*-tetradecanoyl phorbol 13-acetate) response element (TRE) of the regulatory sequences of the wide arrays of different cellular and viral genes.^{4,5} In addition to tumor promoters, the DNA binding of the AP-1 complex to the TRE sequence is rapidly induced by several growth factors, cytokines and oncoproteins, implicated in the proliferation, survival, differentiation and transformation of cells.^{4,6} Because DNA binding is a necessary prerequisite of transactivation, the expression of different proteins of the Jun and Fos family is crucial for the activation of downstream genes regulated by AP-1. AP-1 is also known to control the expression of several target genes that regulate cell cycle (*cyclin D1*, *p16*), differentiation (*myogenin* and *involucrin*), cell survival (*Bcl-2*, *Bcl-xL* and *FasL*), growth factors (*VEGF*), cell adhesion (*VCAM* and *ECAM-1*) and angiogenesis/invasion (*MMPs*, *uPA*, *osteopontin* and *CD44*). As each of the AP-1 family protein is differentially expressed resulting in subtly different functions and in view of heterogeneity in AP-1 complex composition, it is interesting to investigate the overall expression and transactivation pattern of AP-1 proteins in oral carcinogenesis. Dysregulated activation and aberrant expression pattern of AP-1 proteins have been observed in several human cancers including head and neck cancer^{7,8} and oral cancer⁹⁻¹¹ mainly on cell lines and paraffin sections, but,

Key words: oral cancer, human biopsies, carcinogenesis, AP-1, constitutive activation, differential expression

Abbreviations: EMSA: electrophoretic mobility shift assay; HNSCC: head and neck SSC; OCL: oral cancer lesion; OSSC: oral SSC; PCL: precancer lesion; SCC: squamous cell carcinoma

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