Elucidating IFI6 and RSAD2 protein expression in oral squamous cell carcinoma using immunohistochemistry and aptahistochemistry with novel in silico DNA-aptamers

Danial Qasim Butt[1], Nur Asyilla Binti Che Jalil[2], Masitah Hayati Harun[1], Shazana Hilda Shamsuddin[2], Saidi Jaafar[3], Basaruddin Ahmad[4]

[1]Oral Medicine and Oral Pathology Unit, School of Dental Sciences, Universiti Sains Malaysia, 16150, Kota Bahru, Kelantan, Malaysia, [2]Department of Pathology, School of Dental Sciences, Universiti Sains Malaysia, 16150, Kota Bahru, Kelantan, Malaysia, [3]Basic Sciences Unit, School of Dental Sciences, Universiti Sains Malaysia, 16150, Kota Bahru, Kelantan, Malaysia, [4]Biostatistics Unit, School of Dental Sciences, Universiti Sains Malaysia, 16150, Kota Bahru, Kelantan, Malaysia

Track: Research
Theme: Screening and Diagnosis

ABSTRACT

Emerging research identifies interferon-stimulated genes (ISGs) such as IFI6 (Interferon Alpha-Inducible Protein 6) and RSAD2 (Radical S-Adenosyl Methionine Domain-Containing 2) as protumorigenic biomarkers, promoting cancer proliferation and survival. Although their role in apoptotic dysregulation is recognised, their involvement in immune evasion within the oral squamous cell carcinoma (OSCC) tumour microenvironment (TME) remains underexplored, necessitating improved biomarker detection technologies. This study examines IFI6 and RSAD2 protein expression in cancer and immune cells (neutrophils, plasma cells, and lymphocytes) within the OSCC TME using immunohistochemistry (IHC) and aptahistochemistry (AHC) with in silico-derived DNA aptamers. It also designs, characterises, and validates these aptamers for potential AHC application. IFI6 and RSAD2 expression were analysed in OSCC (n=23) and healthy (n=7) tissues via IHC and AHC. Correlations with cancer, immune cell presence, and histological grades were statistically assessed. DNA aptamers were designed in silico, with binding interactions characterised via molecular docking, PyMOL, and PLIP, while molecular dynamics simulations in GROMACS evaluated complex stability (RMSD) and aptamer flexibility (RMSF). Stem-hairpin loop DNA aptamers (35–50 meters) were successfully developed for IFI6 and RSAD2. Among them, 35- and 50-mer IFI6 and 35- and 45-mer RSAD2 aptamers exhibited high binding affinity (-15.7 to -18.7 kcal/mol), strong hydrogen bonding (<4 Å), stable RMSD (<0.4 nm), and flexible loop regions (RMSF >0.4 nm). IHC and AHC analyses revealed significant IFI6 and RSAD2 expression in OSCC tissues, while absent in healthy samples ($p<0.05$). Expression levels were markedly elevated in poorly differentiated tumour grades and tumour-associated immune cells ($p<0.05$). This study confirms IFI6 and RSAD2 expression in OSCC cancer and immune cells, correlating with higher tumour grades. These findings support their potential as prognostic biomarkers in the TME. The in silico-derived DNA aptamers demonstrate strong binding characteristics and hold promise for AHC-based OSCC detection, offering a cost-effective and reliable diagnostic alternative.

Keywords: IFI6, RSAD2, OSCC, Tumour Microenvironment, In Silico-derived DNA-Aptamers