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# Oral cancer cell lines can use multiple ligands, including Fas-L, TRAIL and TNF-α, to induce apoptosis in Jurkat T cells: Possible mechanisms for immune escape by head and neck cancers

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**Summary** Some cancer cells can induce apoptosis in tumour infiltrating cytotoxic T cells as a means of escaping immune destruction. This study examined the expression of the apoptosis-inducing ligands, Fas-L, TRAIL and TNF-α, on three representative oral squamous cell carcinoma (OSCC) cell lines, TR146, SCC25 and CAL27 and investigates the contribution of these ligands to tumour cell killing of Jurkat T cells in vitro. All three cell lines were able to induce apoptosis in Jurkat T cells to varying degrees. The TR146 cell line predominantly killed Jurkats via the well known Fas-L/Fas mediated pathway. Although TR146 also expressed low levels of TRAIL and TNF-α, these did not contribute significantly to TR146 killing of Jurkats. In contrast, the CAL27 cell line expressed little if any Fas-L but was still able to kill Jurkats effectively via an almost exclusively TRAIL mediated mechanism. The SCC25 cell line expressed significant levels of all three ligands but we were unable to significantly inhibit killing of Jurkats by blocking any one pathway with antibodies. SCC25 may use a combination of mechanisms to kill Jurkats and switch between them to compensate when one mechanism is blocked. We found that stimulation with interferon-γ (IFN-γ) induced or increased the expression of apoptosis-inducing

*Abbreviations*: FITC, Fluorescein-isothiocyanate; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; ICC, Immunocytochemistry, IFN-γ, Interferon-γ, OSCC, Oral squamous cell carcinoma; PBS, Phosphate buffer solution; PCR, Polymerase chain reaction, PI, Propidium iodide; RT-PCR, Reverse transcription-polymerase chain reaction; SDS-PAGE, Sodium dodecyl sulphate gel electrophoresis; TNF-α, Tumour necrosis factor-α; TRAIL, TNF-related apoptosis-inducing ligand, WHO, World Health Organisation.

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ligands on OSCC as well as the killing of Jurkat T cells. Not only did IFN-γ increase killing of Jurkats, but it changed the contribution of the Fas-L, TRAIL and TNF-α mediated mechanisms to the killing of Jurkat T cells by the different cell lines. These mechanisms if reproduced in vivo, could confer survival advantage on OSCC by enabling them to kill tumour invading cytotoxic lymphocytes and evade immune destruction.


## Introduction

The development of malignant cells is probably a comparatively common occurrence. However, the bodies innate and immune defence mechanisms are well adapted to detect and destroy developing malignant cells. In particular cytotoxic T lymphocytes (CTL) play an important role in identifying malignant cells and inducing their apoptosis.[1,2] CTL recognise antigenic peptides associated with malignant cells in the context of major histocompatibility complex molecules (MHC) and then destroy them without damaging adjacent normal cells that do not express the antigen.[3] Although CTL can induce apoptosis in target cells by the release of soluble factors such as perforin, killing of tumour cells is thought to occur mainly via CTL cell surface ligand interaction with specific receptors on the tumour cell.[4] These ligands, all members of the TNF family, include Fas-L (CD95-L), TNF-α and TRAIL and they interact with the TNF-receptor family molecules, Fas (CD95), TNF-R1 and TRAIL-R1/TRAIL-R2 respectively.[2,4–6] Ligand binding of receptor induces intracellular activation of the caspase system leading to apoptotic cell death.

The receptors are expressed widely on most cells in the body, including CTL, whereas ligand expression is almost exclusively restricted to CTL.[7] So effective are CTL in destroying tumour cells, that those more successful tumours that have found means to survive are often found to have acquired means of subverting or escaping these mechanisms. In particular, some tumours have acquired the ability to express Fas-L, and other apoptosis-inducing ligands, and this makes them capable of inducing apoptosis in CTL. This ability to kill the CTL that should be killing them has been called ''the Fas counter attack''.[8–10] Since the Fas counter attack was first proposed as a hypothesis, considerable evidence has accumulated to demonstrate that some cancers, including melanomas, hepatocellular carcinomas, gastric adenocarcinomas, oesophageal and other cancers, can mount a counter attack against tumour infiltrating lymphocytes[4,10–12] and this effect has been replicated in vitro using killing of Jurkat T cells by tumour cell lines such as the breast adenocarcinoma cell line MCF-7 and the colorectal adenocarcinoma cell line HT-29.[13,14]

The ability to mount this type of counterattack on CTL, confers considerable survival benefit on the tumour. Not only does it result in the death of CTL that might otherwise kill the tumour cells, but it may also result in immune tolerance to the tumour, which effectively becomes a site of immune privilege.

The aim of this study was to investigate if squamous cell carcinoma cell lines derived from the oral cavity (TR146, SCC25 and CAL27) can mount a counter attack against Jurkat T cells, the most commonly used target for investigating these interactions,[13–15] either through the expression of Fas-L or the alternative apoptosis-inducing ligands TNF-α or TRAIL.

## Materials and methods

### Cell culture

The OSCC cell line, TR146, (a gift from Cancer Research UK) is derived from the buccal mucosa and cultured in DMEM. The OSCC cell lines, SCC25 and CAL27, derived from the tongue (ATCC) were cultured in DMEM F-12 media containing Glutamax, and pyridoxine, including hydrocortisone (0.4 μg/ml), and RPMI 1640 medium containing 25 mM HEPES buffer and L-Glutamine, respectively. The MCF-7 breast adenocarcinoma cell line (ATCC) is derived from the mammary gland, and cultured in MEM media containing non-essential amino acids and sodium pyruvate (1 mM). The HT-29 colorectal adenocarcinoma cell line (ATCC) derived from the colon was maintained in DMEM. The acute T cell leukaemia cell line, Jurkat, was obtained from ECACC and cultured in RPMI 1640 medium containing 25 mM HEPES buffer and L-Glutamine. FCS (10%), Fungizone (2.5 μg/ml), streptomycin (50 μg/ml) and penicillin (50 μg/ml) was added to each bottle of media. Cells were incubated in 5% $CO_2$, 95% air atmosphere at 37 °C, and the medium renewed every 2–3 days.

### Antibodies

The monoclonal $IgG_1$ Fas-L antibody G247-4, (1:25) (BD Biosciences), anti-human TRAIL and anti-human TNF-α monoclonal antibodies (1:100) (R&D) were used in western blot analysis experiments. The monoclonal activating Fas antibody, CH-11, was used in Fas antibody-induced Jurkat apoptosis experiments (0.25 μg/ml) (MBL) and neutralising Fas antibody, ZB-4 (500 ng/ml), was used in Fas antibody-induced Jurkat apoptosis and co-culture experiments (MBL). Also used in co-culture experiments were the monoclonal Fas-L neutralising NOK-1 (2.5 μg/ml) antibody (BD Biosciences) and anti-human TRAIL and anti-human TNF-α monoclonal antibodies (2.5 μg/ml) (R&D).

### RT-PCR

Total RNA was isolated from cell culture flasks using RNA-bee (Biogenesis), purified using chloroform, precipitated using cold isopropanol, washed using 75% ethanol and resuspended in 20 μl DEPC $H_20$. Single stranded cDNA primed with oligo dT was prepared from 5 μg total RNA using Superscriptase II RT (Invitrogen). Each aliquot was subjected to PCR with DyNAzyme II DNA Polymerase (Finnzymes) using specific GAPDH primers (sense: ACCACAGTCCATGCCATCAC