EGF-SubA is cytotoxic to laryngeal SCC cells

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Introduction
EGF-SubA is a novel cytotoxic drug that comprises a bacterial endotxin (SubA) covalently bound to EGF to permit targeting of EGF-expressing cells. The SubA moiety clears GFPBR, a key component of the unfolded protein response which is induced as part of a survival response under conditions of stress (e.g. hypoxy/hypoglycaemia) commonly observed in solid tumours.

Analysis of GRP78 expression on a TMA with 196 patient samples indicates that GRP78 is up-regulated in squamous cell carcinoma of the head and neck (SCCHN) and thus we hypothesise that EGF-SubA might prove a potent inhibitor of SCCHN cells.

Abstract
1. The effect and mechanism of action of EGF-SubA on laryngeal (SCC) cells. EGF-SubA is a novel cytotoxic drug that promotes cleavage of GRP78 as a key component of the unfolded protein response.
2. The interaction of EGF-SubA with clinically relevant modulators: cisplatin and gamma irradiation in LSCC cells.

Method
In vitro cytotoxicity was determined for a panel of 7 LSCC lines of varying p53 status using MTT assays. For studies of EGF-SubA combined with cisplatin or gamma irradiation, apoptosis was also determined by flow cytometry. Clonogenic assays were used to determine the effect of combining EGF-SubA with gamma radiation.

Results
EGF-SubA is cytotoxic to LSCC cells at picomolar concentrations, regardless of p53 status in lines derived from primary tumour sites and from metastases. The cytotoxic effects of EGF-SubA are not rapid, limited apoptosis is observed, and thus it is not clear whether this depends upon apoptosis.

EGF-SubA acts as a radiosensitising agent when used in combination with gamma radiation producing a significant (p<0.03) reduction in the surviving fraction at 26Gy. Furthermore, EGF-SubA evoked at least an additive cytotoxic effect when used in combination with cisplatin. Flow cytometry demonstrated that in contrast to EGF-SubA alone, both combined treatments induced apoptosis.

Conclusion
In vitro EGF-SubA is highly cytotoxic to LSCC cells at picomolar concentrations and whilst the mechanism of cell death of EGF-SubA alone is unclear, combinations of EGF-SubA with genotoxic agents potently induce apoptosis.

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Materials and Methods
LSCC cell lines (n=7, courtesy of the University of Michigan [i.e. UMSCC]) were examined by cell counting and MTT assay for viability, clonogenic assay to determine radiosensitivity, and by flow cytometry with propidium iodide (PI) and annexin V to examine the cell cycle (not shown) and to detect cell death and apoptosis.

Figure 1: EGF-SubA is cytotoxic to all UMSCC cell lines at picomolar concentrations.

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Cisplatin resistant SCC12A and SCC17A were also probed and demonstrated synergism of EGF-SubA with cisplatin.

Figure 2: Isobolograms for UMSCC 5, 12 and 17A demonstrating synergism of EGF-SubA and cisplatin.

EGF-SubA acts as a radiosensitiser in vitro at picomolar concentrations.

Conclusion
In vitro EGF-SubA is highly cytotoxic to LSCC cells at picomolar concentrations independently of tumour p53 status. It is also synergistic in combination with cisplatin and acts as a radiosensitiser. Whilst the mechanism of cell death of EGF-SubA alone is unclear, combinations of EGF-SubA with genotoxic agents potently induce apoptosis.

In other studies we have found that GRP78 (the target of EGF-SubA) is up-regulated in most SCCHNs. Combining this with evidence from murine xenograft models showing systemic tolerance of EGF-SubA, we propose to perform further toxicity studies in vivo to examine the therapeutic index/potential of this novel reagent and its effectiveness against SCCHN.

EGF-SubA acts as a radiosensitisier in LSCC lines

Clonogenic assays show that EGF-SubA at 3-10pM acts as a radiosensitising agent reducing the 50% survival fraction by at least 30% in four UMSCC lines (G0.02 to 0.03) shown in Figure 4.

Figure 4: UM SCC cells were pre-treated for 24h with EGF-SubA at either 3 or 10pM or drug vehicle control and then exposed to pre-irradiation at 0, 1, 2, 4 or 8 Gy by an irradiation. Clonogenic assays were established to allow for colony formation over a time period of two to three weeks. A colony was defined as equal to or more than 50 cells in order to allow for at least five cell doubling times.

Results part 2

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