Neurons increase the migration speed of head and neck squamous cell carcinoma cells through a HER3-dependent mechanism.

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INTRODUCTION

Perineural invasion (PNI) in squamous cell carcinoma of the head and neck (HNSCC) has been associated with poor outcomes, including increased local recurrence and decreased survival. The mechanism behind PNI has yet to be elicited.

It has been shown that HER3 is overexpressed in certain HNSCC cell lines. The chief ligand for HER3, neuregulin (NRG), has been shown to be secreted by both neurons and Schwann cells. Thus, the interaction between HER3 expressed on HNSCC cells and NRG secreted from neurons could be responsible for PNI.

Cellular migration plays a large role in invasion, and it can reliably be measured in vitro using a migration assay. The purpose of this study is to determine if an interaction between HNSCC cells and neurons affects migration of HNSCC and to elicit the role of HER3 in this interaction.

We hypothesize that an interaction between HER3 and NRG will cause HNSCC cells to migrate faster. Similarly, we hypothesize that by blocking HER3 with an antibody or knocking down HER3 with shRNA, the effect of faster migration by HNSCC cells will diminish.

METHODS AND MATERIALS

24 mouse dorsal root ganglia (DRGs) were dissected from 3-4-week-old female Foxn1nu mice. DRGs were dissociated into individual neurons using standard protocol reagents including papain, collagenase, and dispepsin. The neurons were cultured in 500uL of DMEM/F12 media containing fetal bovine serum for three days around the outside of a co-culture plate that contains a culture insert. (Figure 1)

In each well of the insert, 40,000 FaDu cells were placed in 80uL of DMEM media. After the FaDu cells had attached to the plate, the insert was removed, leaving a ~500um gap between cell columns. (Figure 1) Photos were taken every 4 hours over a 12 hour period, and the area of the gap was measured.

There were four experimental conditions: 1) FaDu cells with no neurons, 2) FaDu cells with neurons, 3) Either FaDu cells with the HER3 antibody AV203 at 10ug/mL or FaDu cells with HER3 knocked down via shRNA with no neurons, and 4) Neurons with either FaDu cells with AV203 or shRNA against HER3 with neurons.

Percent decrease in gap area was used to quantify cellular migration. Groups were compared by ANOVA.

RESULTS

At baseline, FaDu cells took nearly 24 hours to close the gap (not shown).

The presence of neurons significantly increased the speed of migration of FaDu cells (61.9% gap closure; Neurons vs. 25.4% Control, p<0.001).

Adding the HER3 antibody AV203 or knocking down HER3 with shRNA did not affect baseline migration speed of FaDu cells (15.5% AV203 vs. 25.4% control, p>0.05; 11.7% shHER3 vs. 15.7% control, p>0.05).

Only in the presence of neurons did adding 10ug/mL AV203 show a significant decrease in FaDu cell migration (61.9% Neurons vs. 43.3% Neurons/AV203, p<0.001). Similarly FaDu/shHER3 cells migrated more slowly than FaDu cells only in the presence of neurons (47.6% Neurons vs. 33.2% Neurons/shHER3, p<0.01).

CONCLUSIONS

We have shown that FaDu cells respond to the presence of neurons by increasing their migration speed.

By blocking HER3 with AV203, the effect of neurons on migration is significantly diminished. This effect is reproduced when knocking down HER3 via shRNA.

Since AV203 and FaDu/shHER3 cells migrated at the same speed as control cells, baseline FaDu cell migration is not dependent on HER3. However, FaDu the increase in migration speed in response to neurons is at least partially dependent on HER3.

Cellular migration is an important aspect of the invasive process in cancer. Since our study has shown that FaDu cells migrate more quickly in the presence of neurons in vitro, it may follow that HNSCC also migrates faster and invades more aggressively in the presence of a nerve in vivo.

Our study has shown that migration in response to neurons is dependent on HER3 in vitro. Therefore, it is possible that in certain patients, a HER3 antagonist may prevent or lessen the degree of PNI in vivo, though this idea needs to be explored further.

REFERENCES