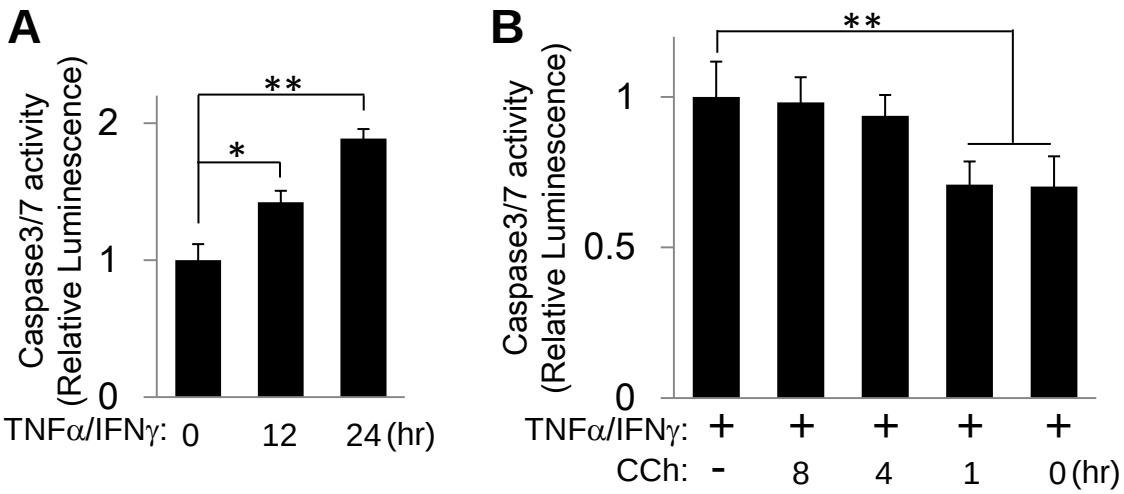
Muscarinic type 3 receptor induces cytoprotective signaling in salivary gland cells via EGFR transactivation

Mikihito Kajiya, Isao Ichimonji, Christine Min, Tongbo Zhu, Jin Jun-O, Qing Yu, Soulafa Almazrooa, Seunghee Cha, and Toshihisa Kawai

Molecular Pharmacology



Supplemental Fig. 1. Relation between proinflammatory cyokint and CCh on caspase3/7 activity.

(A) HSG cells were exposed to TNFα (50 ng/ml)/IFNγ (10 ng/ml) for 0, 12, or 24 hr. (B) HSG cells were pretreated with or without CCh (100 μM) for the indicated time. Then the cells were stimulated with TNFα (50 ng/ml)/IFNγ (10 ng/ml) for 24 hr. Caspase3/7 activity was indicated by luminescence activity. Values represent means ± S.D. of three cultures. * $p < 0.05$, ** $p < 0.01$: Values differ significantly (*t*-test).

Supplemental Figure1

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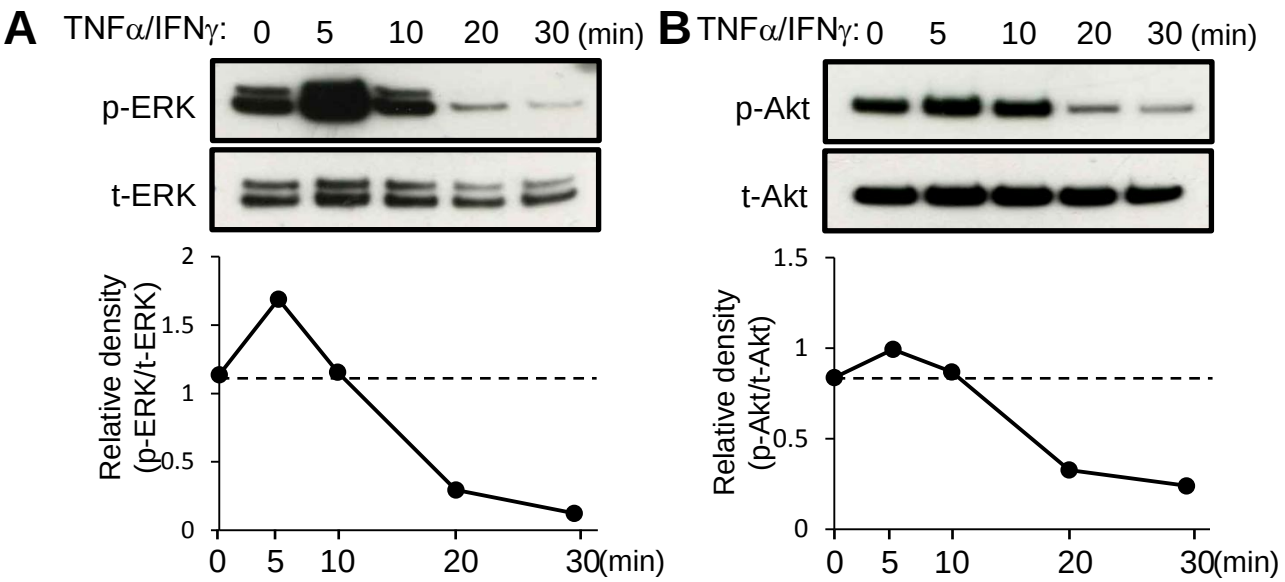

Supplemental Fig. 2. The effect of TNFα/IFNg treatment on ERK and Akt phosphorylation level in HSG cells.

(A and B) HSG cells were exposed to TNFα (50 ng/ml)/IFNγ (10 ng/ml) for the indicated times. The phosphorylated or total ERK (A) and Akt (B) levels were analyzed by immunoblotting. Quantification of the band density was performed by densitometric scanning of each band using NIH image software.

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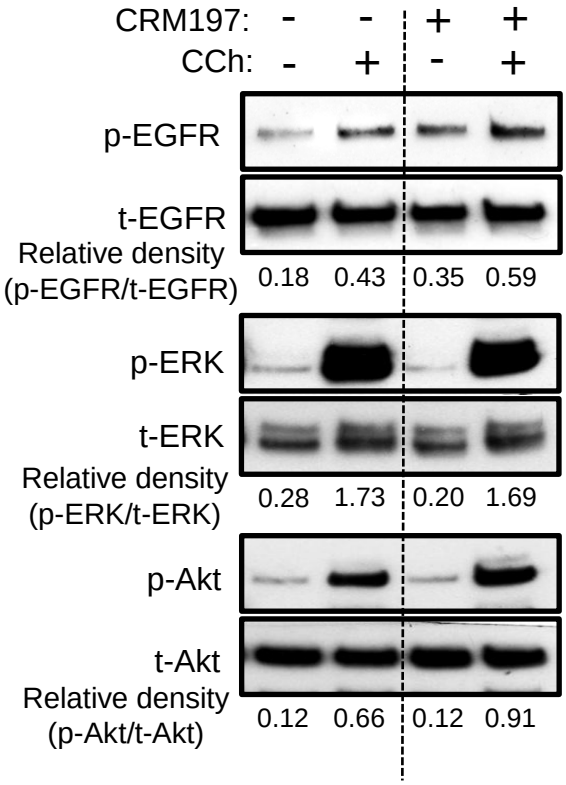

Supplemental Fig. 3. Heparin binding (HB)-EGF inhibitor, CRM197 does not affect the CCh-induced cell survival signaling in HSG cells.

HSG cells were pretreated with or without 1 μg/ml of CRM197 for 30 min and then exposed to CCh (100 μM) for 5 min. The phosphorylated or total EGFR, ERK, and Akt levels were analyzed by immunoblotting.

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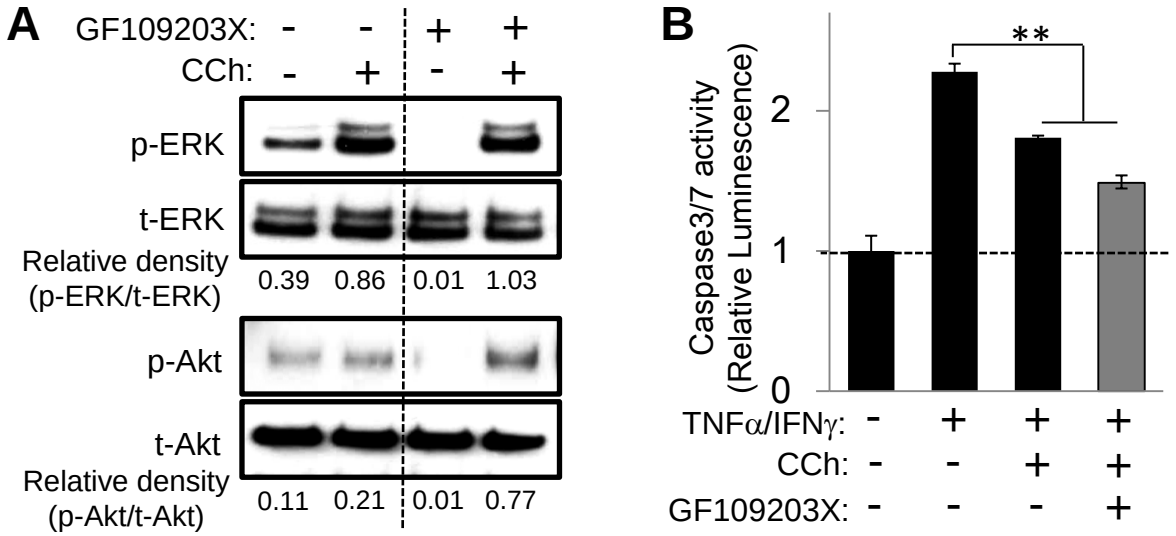

Supplemental Fig. 4. PKC inhibitor, GF109203X does not affect the CCH-induced cell survival signaling in HSG cells.

(A) HSG cells were pretreated with or without GF109203X (1 μM) for 30 min and then exposed to CCh (100 μM) for 5 min. The phosphorylated or total ERK and Akt levels were analyzed by immunoblotting. Quantification of the band density was performed by densitometric scanning of each band using NIH image software. (B) HSG cells were pretreated with or without GF109203X (1 μM) for 30 min. Then the cells were treated with or without 100 μM of CCh in the presence or absence of combined TNFα (50 ng/ml)/IFNγ (10 ng/ml) and incubated for 24 hr. Caspase3/7 activity was indicated by luminescence activity as described in the Materials and Methods section. Values represent means ± S.D. of three cultures. ** $p < 0.01$: Values differ significantly (*t*-test).