Dermacentor variabilis: Tick Saliva Inhibits Osteosarcoma Cell (SaOs) Migration and Invasion

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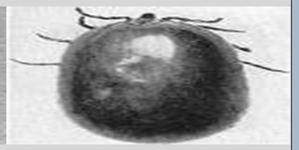
Introduction

Dermacentor variabilis commonly known as the American dog tick belongs to the Family Ixodidae. These hard ticks are ectoparasites which attach to the host for several days and exhibit pool feeding by alternating saliva secretion and blood ingestion. It takes 6-10 days for female ticks to become engorged. Male ticks do not become engorged.

Non-engorged Female Tick



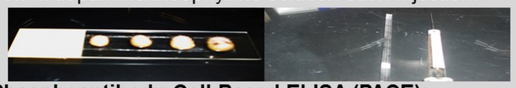
Engorged Female Tick

This study examines the effect of tick saliva on osteosarcoma cell (SaOs) migration, invasion, and colony formation. Osteosarcoma predominately affects rapidly growing bones in adolescents (1) and is the fifth most frequent malignancy in 15-19 year olds (2). Osteosarcoma patients who later develop metastatic disease only have a 20-30% survivorship 10 years after diagnosis (3,4).

Methods

Cell Culture for SaOs: All cells were cultured in alpha minimal essential medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS).

Saliva Collection: Partially engorged female dog ticks (90-350mg) were injected a total of 4 times with 10µl of a 10mM dopamine/theophylline solution each injection.



Phosphoantibody Cell-Based ELISA (PACE): Epidermal Growth Factor (EGF)-activation of ERK and Akt was measured using a PACE assay. SaOs cells were plated at 60,000 cells/cm^2 in 96-well plates. Cells were starved 18-20 hours before experiment in medium with 0.1% FBS. Cells were pretreated for 30 minutes with saliva (see figure legend 1) and experiments were stopped with cold PBS. Cells were fixed (4% formaldehyde), blocked (5% non-fat dry milk or BSA in PBS-Triton(T), and dishes were incubated overnight at 4 ºC with primary antibody (phosphoERK 1:8000 or Akt 1:500). Dishes were washed with PBS-T, incubated with goat anti-mouse or rabbit-HRP 1:1000, washed, and developed with 1-step Ultra TMB ELISA substrate.

Blind-well Assay: SaOs cells (100,000 cells/ml) were suspended in media and pretreated with saliva for 30 minutes (see figure legend 2). The bottom well of the chemotaxis chamber was loaded with 40µl media only or media and 10% FBS (chemoattractant). An 8µm filter was placed over the bottom well and the top well was screwed into place then loaded with 200µl of cell suspension containing the vehicle control or the experimental treatment . After 6 hours of incubation at 37℃ in 5% CO_2, cells that migrated across the filter were fixed (75% methanol), stained (crystal violet), and counted using a light microscope.

Chemotaxis Plate Assay: SaOs cells (510,000 cells/ml) were suspended in media and pretreated for 30 minutes (see figure legend 3). The bottom well of the plate was loaded with 29µl media and 10% FBS, and 50µl cell of suspension containing the vehicle control or the experimental treatment was added to the top of the filter. The cells were incubated, fixed, stained, and counted as previously mentioned.

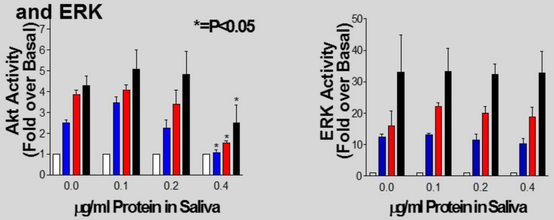
Invasion Assay: Invasion assay was performed using a BD Biocoat matrigel 24-well plate invasion chambers. SaOs cells (50,000 cells/ml) were suspended in media and pretreated with saliva for 30 minutes (see figure legand 4). The bottom chamber was loaded with 0.75ml media and 10% FBS (chemoattractant) and the upper chamber was loaded with 0.5ml of cell suspension containing the vehicle control or experimental treatment. After 36 hours of incubation, cells that migrated across the matrigel and/or filter were fixed, stained, and counted as previously mentioned.

Colony Formation Assay: SaOs cells (500,000 cells/ml) were suspended in a 0.3% agarose solution continuously exposed to saliva (see figure legend 4) overlying a 0.7% agarose solution. After 2 weeks of incubation at 37℃ in 5% CO_2, the colonies were stained (crystal violet) and counted using an inverted microscope.

Statistical Analyses: Data presented are ±SEM of 3-4 experiments performed over ≥3 passages. Statistical significance was determined by 1-way ANOVA, Student-Newman-Keuls, and Dunnett's multiple comparison post-tests using GraphPad Prism v.3.02 for Windows.

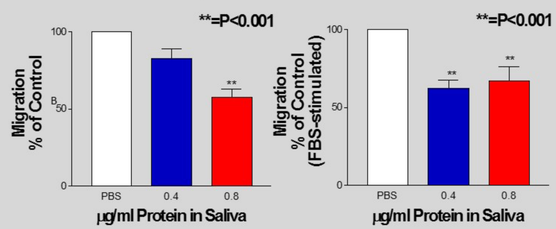
Results

Figure 1. Effect of Tick Saliva on EGF-Activation of Akt and ERK



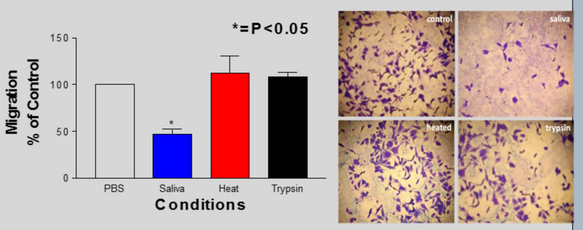
Cells were pretreated for 30 minutes with PBS or saliva containing a protein concentration of 0.1, 0.2, or 0.4µg/ml (n=4). Saliva with a protein concentration of 0.4µg/ml significantly decreased Akt activity, however, saliva had no significant effect on ERK activity

Figure 2. Effect of Tick Saliva on Basal and FBS-Stimulated Cell Migration



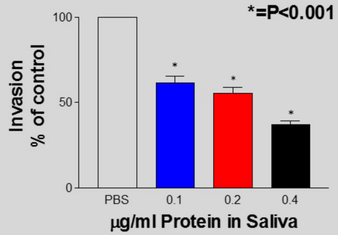
Cells were pretreated for 30 minutes with PBS or saliva containing a protein concentration of 0.4 or 0.8µg/ml (n=4). Saliva with a protein concentration of 0.8µg/ml significantly inhibited basal and stimulated migration. Saliva with a protein concentration of 0.4µg/ml was also inhibitory to stimulated migration.

Figure 3. A Protein(s) in Tick Saliva Is Responsible for Migration Inhibition



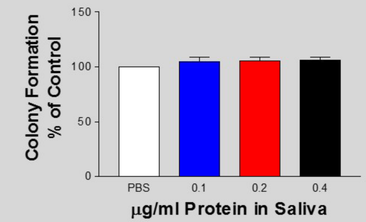
Cells were pretreated for 30 minutes with PBS, saliva containing a protein concentration of 0.4µg/ml, saliva heated at 75 ºC, saliva treated with trypsin at a volume of 1:2 (n=3). Denaturing the proteins in tick saliva with heat and trypsin returned the stimulated cell migration observed in the vehicle control.

Figure 4. Effect of Tick Saliva on Stimulated Invasion



Cells were pretreated for 30 minutes with PBS or saliva containing a protein concentration of 0.1, 0.2, or 0.4µg/ml (n=3). Saliva containing protein concentrations of 0.1, 0.2, and 0.4µg/ml significantly inhibited cell invasiveness.

Figure 6. Effect of Tick Saliva on Colony Formation.



Cells were continuously exposed to PBS or saliva containing a protein concentration of 0.1. 0.2, or 0.4µg/ml for 2 weeks (n=3). Saliva had no significant effect on colony formation.

Discussion

Our data indicate that a protein(s) in tick saliva inhibits the migration and invasiveness of an osteosarcoma cell and suggest that suppression of Akt signaling may be involved. However, it did not indicate any effect on ERK signaling or colony formation.

Since metastasis of primary tumors often occurs in cancer, our data suggest the possibility of therapeutic agent(s) in tick saliva.

References

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