



Role of *Helicobacter pylori* in gastric carcinoma

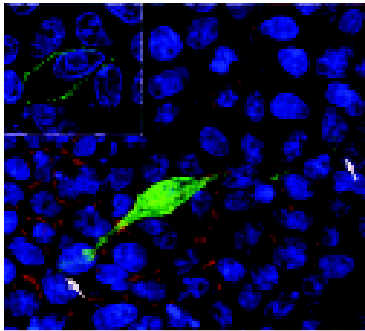


Fig. 1

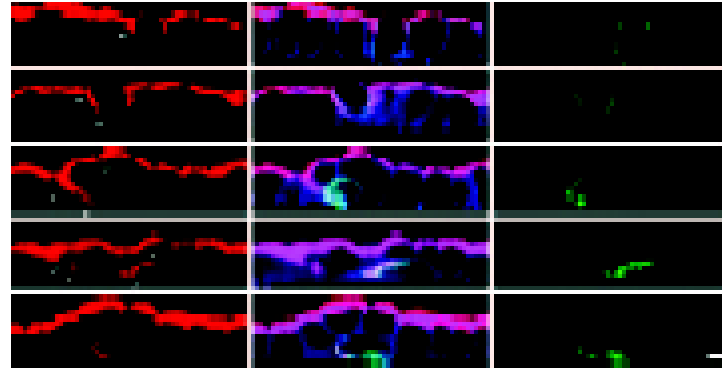


Fig. 2 (scale bar: 10µm)

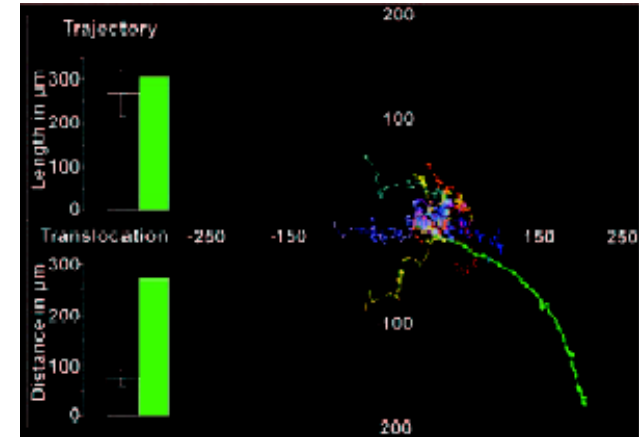


Fig. 3

Dr Fabio Bagnoli and colleagues from Stanford University in California and Chiron Vaccines in Italy are involved with the study of *Helicobacter pylori*, a spiral shaped bacterium that lives near the epithelial cells of the human stomach. Within a few days of infection, gastritis occurs, and in some people this eventually results in ulcers or stomach cancer.

In the work described here, the researchers were interested in a protein called CagA which is injected by the adhered *H. pylori* into the host epithelial cells. This effector protein interferes with the host cell signaling pathways, that when altered are associated with oncogenic cell transformations. To explore CagA's biological properties, the researchers used canine epithelial cells (MDCK) expressing GFP-tagged-CagA-protein for quantitative confocal immunofluorescence and for time-lapse analysis.

The first shown effect of CagA was phenotype alteration. 0.5µm confocal optical sections of MDCK monolayers expressing GFP-CagA were captured and imported into **Volocity Visualization** for fast 3D analysis. Fig. 1 is a **Volocity** image view of the monolayer with one elongated cell (green) spanning about nine cell diameters (arrows). Nuclei are stained in blue.

Fig. 2 is a montage of **Volocity** 3D reconstructions of polarized MDCK monolayers expressing GFP-CagA (green) and stained for F-actin (blue). The monolayers were also stained for the apical membrane glycoprotein gp135 (red) in order to find out if CagA had an effect on cell polarity that could explain the morphological changes observed in Fig. 1. The images show a clear mislocalization of gp135 in the basolateral membrane. Loss of polarity and cell elongation suggested that CagA may cause cell-cell adhesion disruption and induce cell migration. To test this hypothesis, a time-lapse experiment was performed where images were acquired every 10 minutes for 6-16h. Fig. 3 uses the charting functionality of **Volocity Classification**, where trajectories of control cells (color-coded) and CagA expressing cells (green) are displayed, showing that CagA expressing cell translocated four times further from their point of origin, confirming a loss of cell adhesion. Bar graphs indicate that the average trajectory length of control cells (gray) was similar to CagA cells (green), indicating that all cells moved at similar speeds. However, the translocation distance of CagA cells was four times longer because these cells moved without adhesive constraints.

Volocity Visualization and **Classification** allowed researchers to easily perform a wide range of analysis from visualization of proteins and cell morphology changes in 3D to automatic tracking and charting of cell movements.

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