

Abstract #3455

MATERIALS & METHODS

- A375 melanoma cells (ATCC)
- HoloMontitor M4
- Hstudio[™] M4 version 2.6.3
- Recombinant, DetoxiGel purified human cystatin C

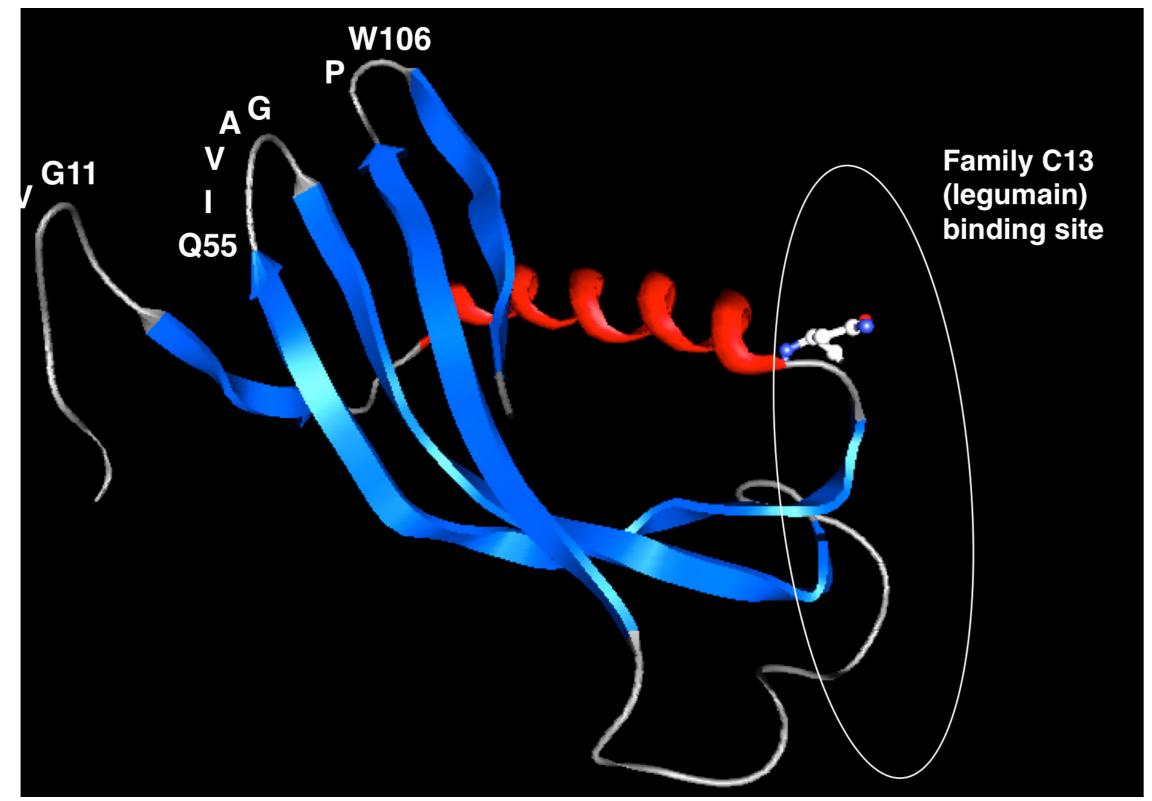


Fig. 1. Cystatin C is a small secreted protein, produced by most cells in the body and found in significant quantities in all body fluids. It is the major inhibitor of extracellular cysteine protease activity in mammals because of its rapid binding to virtually all known cysteine proteases of families C1 (cysteine cathepsins) and C13 (mammalian legumain).

AIM

The purpose of the present study was to elucidate possible direct effects of cystatin C on melanoma cells, by use of holometric imaging to analyze the cells in real-time. Human A375 melanoma cells were cultured with and without addition of physiological quantities of cystatin C added to the culture medium (1 μ M). The cultures were monitored for up to 3 days in a standard incubator using a HoloMonitor M4 instrument.

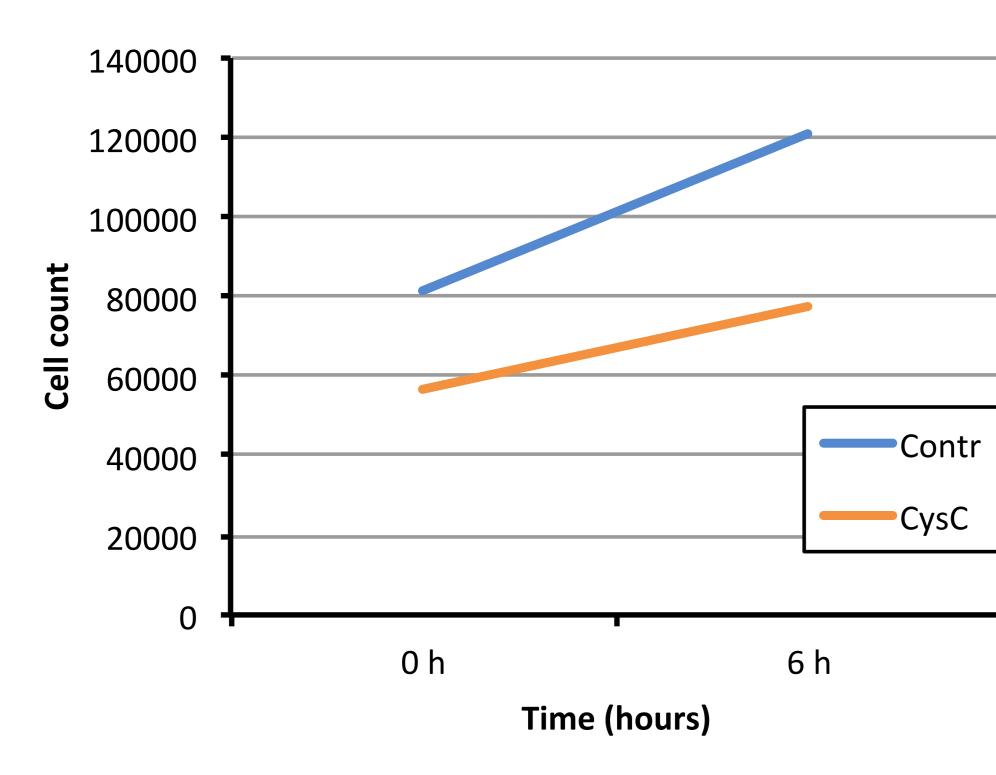


Fig. 5. Short-term effect of cystatin C on the proliferation of A375 cells. [Analysis by Hstudio, of 10 images (approx. 300 cells) from triplicate cultures of cells grown in medium with or without 1 µM cystatin C (variation ±15%)]

Cystatin C decreases proliferation of melanoma cells by affecting mitosis length

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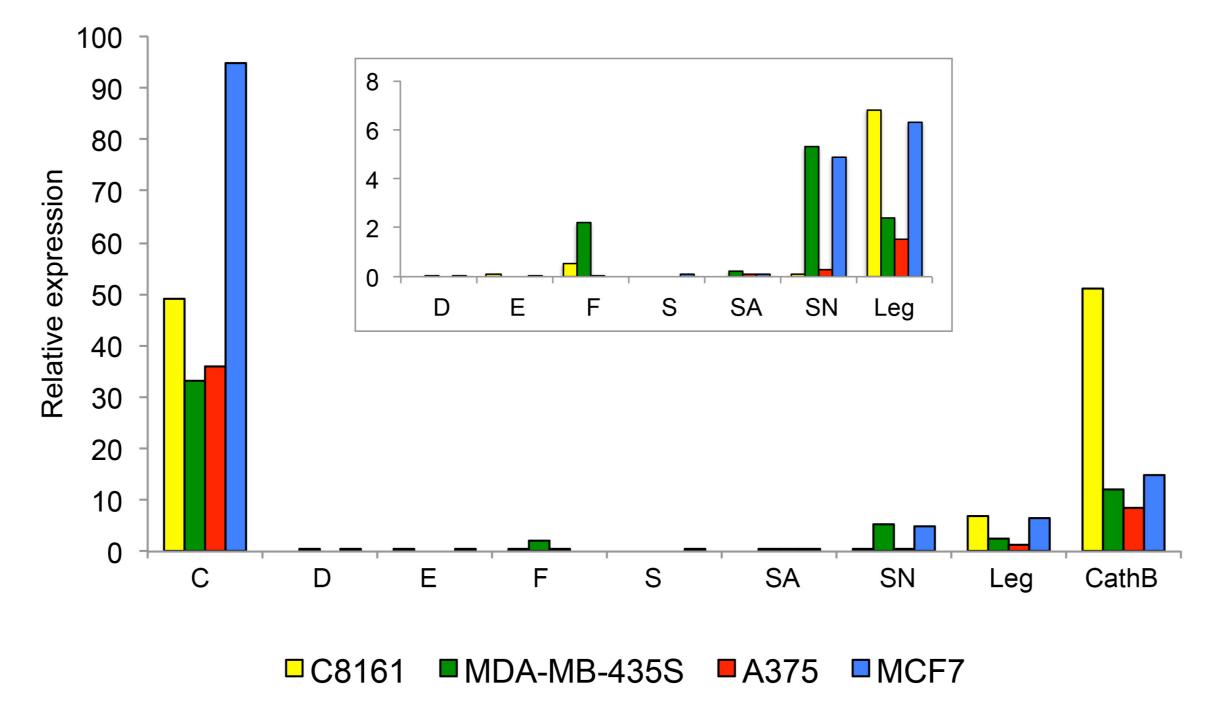


Fig. 2. Cystatin C is the only of the 7 secreted type 2 cystatins that is abundantly expressed in melanoma cells. [mRNA levels were measured by qRT-PCR and related to 18S rRNA levels (ratio multiplied by a factor of 10e6)]

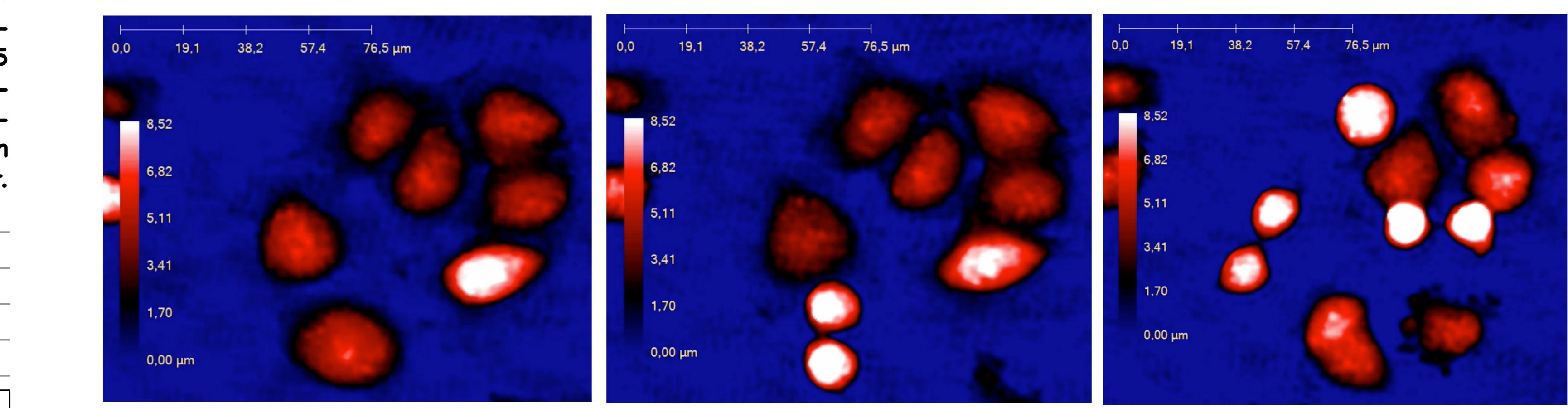


Fig. 6. Visualization of A375 cells in the HoloMonitor. [A375 cells; films generated from images captured every 5 mins; left and right images 24 hrs apart]

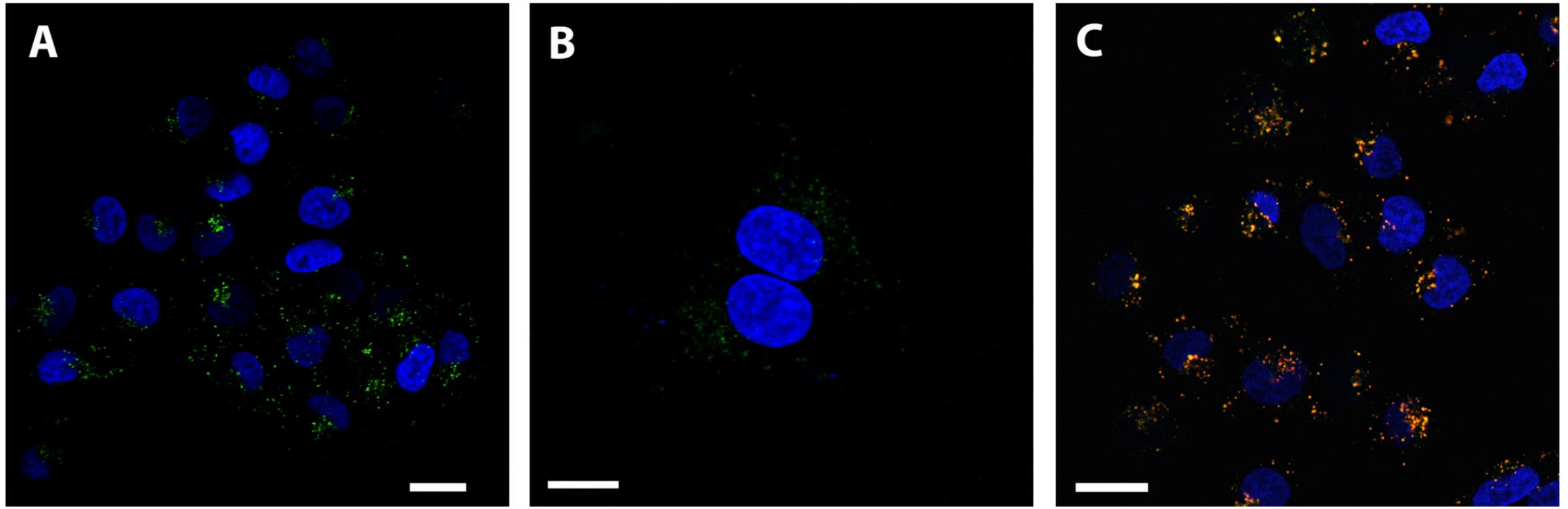
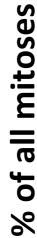


Fig. 3. Cystatin C is internalized into perinuclear vesicles in melanoma cells. (A) A375 cells, cystatin E/M green, (B) MDA-MB-435S cells; cystatin E/M green; (C) Co-localization of cystatin C (red) and E/M (green) in endolysosomal compartments. [Analysis by confocal microscopy; DAPI blue; scale bar equals 20 µm in A and C, 10 µm in B]



RESULTS The analyses revealed qualitative differences in the normal behavior of the A375 cells under study as a result of cystatin C addition. The motility of the cells was significantly affected. The mitosis phase was considerably prolonged in cells grown in cystatin C containing medium, indicating that the proliferation-inhibiting effect of the protease inhibitor may be through its direct action on proteolytic processes in the mitosis phase.



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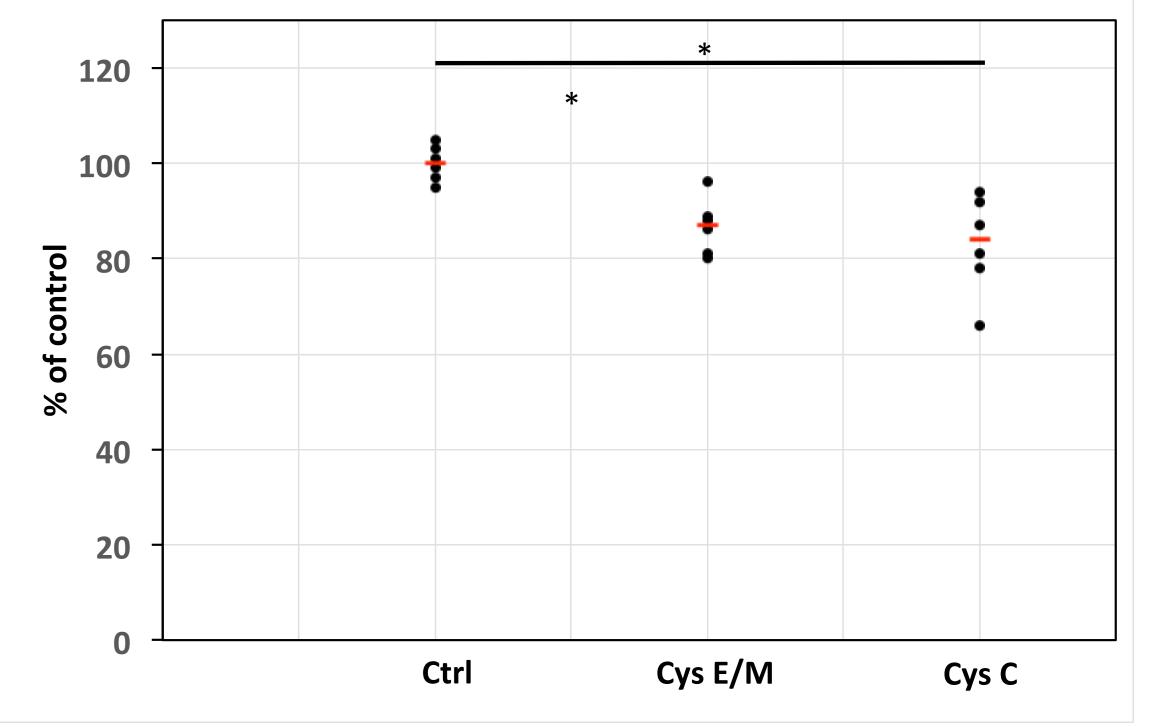


Fig. 4. Effects of cystatin Cuptake on migration of melanoma cells. A375 cells were starved and then incubated for 72 h with and without 5 μ M cystatin E/M or 5 μ M cystatin C in transwell filters, with or without Matrigel coating.

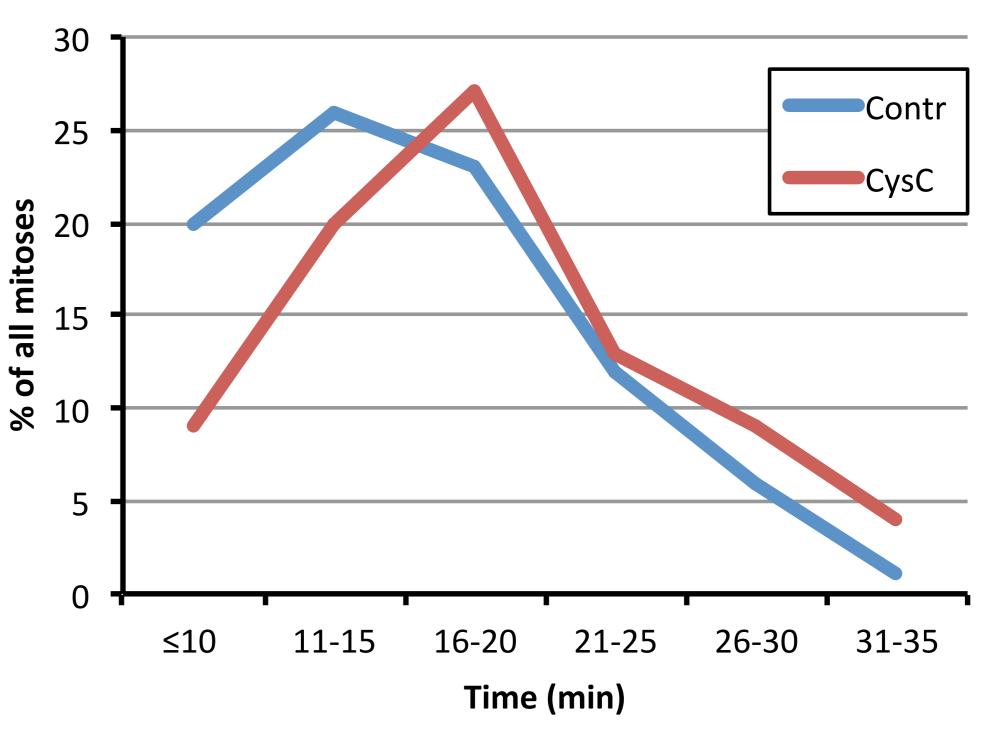


Fig. 7. Measurement of mitosis length in A375 cells, cultured with or without 1 μ M cystatin C. [Individual mitotic cells were identified manually and tracked through the films from images captured every 5 mins (600 mitoses tracked)]