ADAPTIVE RESPONSE OF HELA CELLS UNDER SHEAR STRESSES IN MICROCONFINEMENT THROUGH THE AUTOPHAGIC PATHWAY

J. Das, A.K. Praveenkumar, B. Roy, S. Chakraborty and T.K. Maiti
Indian Institute of Technology Kharagpur, INDIA

ABSTRACT

We report that human cervical cancer (HeLa) cells respond to fluid shear stresses by upregulating autophagy. We used a biomimetic microfluidic platform to assess the effect of fluid shear stresses in microconfinement, on autophagy induction in HeLa cells. Cells responded to laminar, oscillatory and pulsatile flows by showing increase in autophagosome formation that peaked around 10, 20 and 10 minutes respectively and reverted to basal levels within 30 minutes of flow. This autophagic response might be the secret of how cancer cells evade the wrath of shear in circulation during metastasis and thus a key target for anticancer therapy.

KEYWORDS: shear stress, microconfinement, HeLa cells, autophagy

INTRODUCTION

In distinct physiological conditions, cells experience various mechanical stresses like shear, torsion, and compression due to haemodynamic forces and solid stresses. While researchers have focused on the biological outputs of cells in biomimetic confinements owing to incipient shear, reports regarding stresses generated on cells in such conditions and the mechanism by which they evade the same are rare. Shear stresses range from 0.5-4 dynes cm^{-2} in the venous circulation to 4-30 dynes cm^{-2} in the arterial circulation [1]. Increase in fluid shear forces could affect cancer cell survival, as only a small portion of circulating tumor cells (CTCs) survive the circulation to generate metastases. Cells have several internal mechanisms to avert stress conditions. Autophagy, a catabolic process that maintains cellular homeostasis and other processes, is known to be upregulated during various cellular stresses. Previously, compressive forces have been reported to induce autophagy in MDA-MB-231cells *in vitro* [2]. However, the impact of shear stresses on autophagy in cells in microconfinements remained unexplored. Thus we endeavoured to study the influence of various forms of shear stress on cellular autophagy and its implication in cell survival. We used Poyldimethylsiloxane (PDMS)-based microconfinements [3] to study the effect of shear stress on HeLa cells that were earlier shown to be mechanosensitive [4].

EXPERIMENTAL

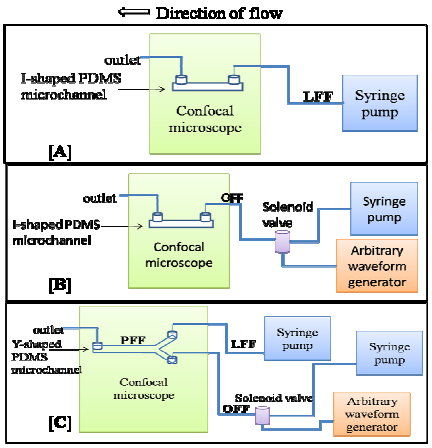

Figure 1: Schematic of experimental setups for the generation of [A]laminar, [B]oscillatory and [C]pulsatile shear stresses. [LFF: laminar fluid flow, OFF: oscillatory fluid flow, PFF: pulsatile fluid flow]

HeLa cells were cultured in sterilized fibronectin-coated I- and Y- shaped biomimetic PDMS microchannels at 37ºC, pH 7.4 and 5% CO_2 environment. For detection of autophagy, the seeded cells were first transfected inside the channels with EGFP-LC3B Addgene plasmid 11546 for transient expression [5]. 24hrs after transfection, various types of flow were applied as shown in Figure 1, with complete media to ensure proper nutritional availability for cells. To expose HeLa cells to physiological shear stresses, laminar, oscillatory and pulsatile shear stresses with peak shear stress of 1 Pa were applied. Magnitudes of shear stresses were selected such that the cells did not get washed away due to flow. Autophagy was detected by monitoring the Green Fluorescent Protein tagged Microtubule-associated protein 1 light chain 3 alpha (GFP-LC3) puncta (autophagosome) formation at different time scales in flow conditions. To determine the fate of a cell undergoing shear stress in the absence of autophagy, transfected cells were pre-treated with 3-Methyladenine (3-MA), an early- autophagy inhibitor and stained with nuclear stain 4',6-diamidino-2-phenylindole (DAPI) to check for apoptosis, a programmed cell death process. Cells with membrane blebbings and fragmented DNA were taken to be apoptotic.

RESULTS AND DISCUSSION

HeLa cells exposed to laminar shear stress of 1Pa showed increase in number of autophagosomes reaching a maximum around 10 minutes of exposure to flow. For oscillatory shear stress, maximum autophagosomes were noted around 20 minutes and for pulsatile shear stress, around 10 minutes, as shown in Figure 2. However, for all three cases, the autophagosome number declined to that of basal levels within 30 minutes of exposure to flow. This indicated that HeLa cells responded to shear stresses by upregulating autophagy and the same was restored to basal levels once the cells adapted to the applied shear stress.

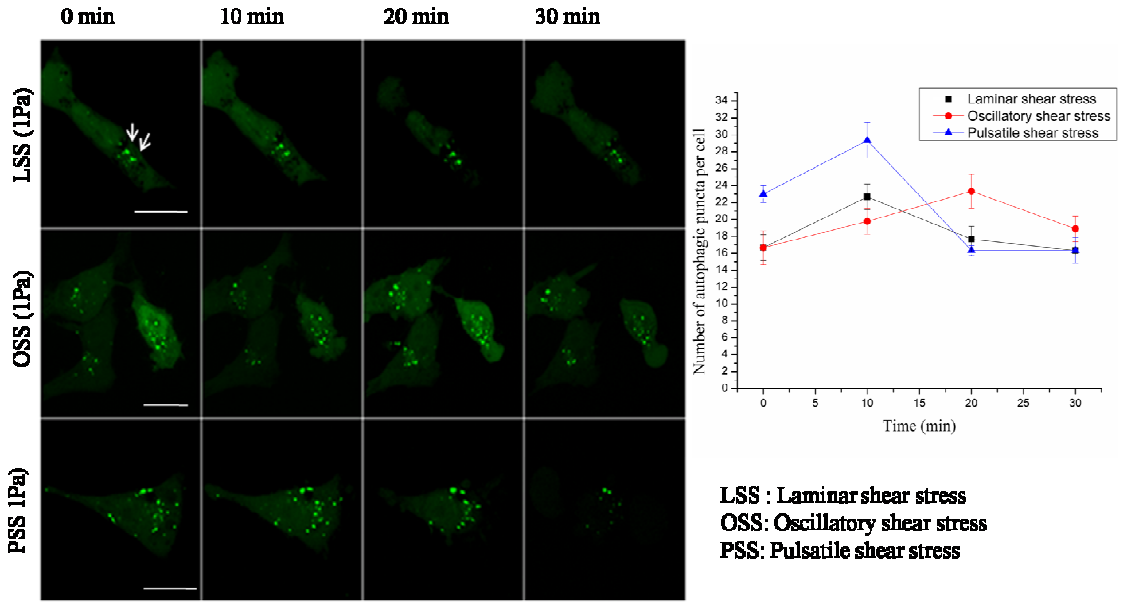

Figure 2: Autophagic state of GFP-LC3 expressing HeLa cells along with quantitative measure of autophagosomes at various time points, under laminar, oscillatory and pulsatile flows. Scale bar represents 10 microns. White arrows indicate autophagosomes. All experiments were repeated thrice and cells with more than at least 15 autophagic puncta were taken into consideration.

HeLa cells pre-treated with an early- autophagy inhibitor, 3-MA and experiencing shear stresses were found to show signs of apoptosis (Figure 3 A-B) within half an hour of flow whereas no such phenomenon was noted for untreated cells. On the contrary, the untreated cells showed increased autophagosome formation (Figure 3 C-F) indicating that autophagy might be a key survival process for HeLa cells experiencing high shear stresses in circulation.

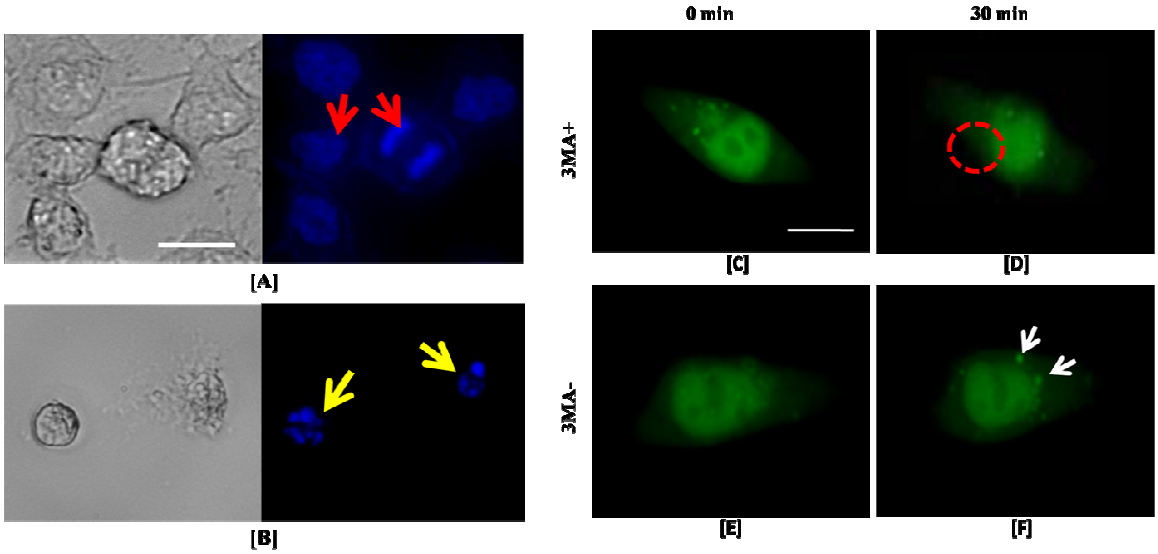

Figure 3: [A] and [B] show phase contrast and fluorescence images of DAPI-stained 3-MA- pre-treated HeLa cells in microchannel showing intact DNA (marked by red arrows) before application of flow and apoptotic DNA (marked by yellow arrows) after exposure to flow. [C] and [D] show a GFP-LC3 expressing HeLa cell pre-treated with 3-MA, before and after 30 minutes of pulsatile fluid flow respectively. [E] and [F] show a similar cell untreated with 3-MA, before and after 30 minutes of flow respectively. Red circle highlights membrane blebbings while white arrows indicate autophagosomes. Scale bars represent 2 microns.

CONCLUSION

Our findings thus suggest that HeLa cells adapt to shear stresses in narrow confinements by resorting to autophagy, as indicated by enhanced autophagosome formation. This mechanically induced autophagic process gets upregulated initially and reverts to basal levels once the cell adapts to the changing physical environment. Thus, this stress adaptive pathway may be a potential target for anticancer therapy.

REFERENCES

[1] M.J. Mitchell and M.R. King, "Fluid shear stress sensitizes cancer cells to receptor-mediated apoptosis via trimeric death receptors", New Journal of Physics, 15, 015008, 18 pp, 2013.

[2] J.S. King, D.M. Veltman and R.H. Insall, "The induction of autophagy by mechanical stress", Autophagy, **7**, 1490-1499, 2011.

[3] W.J. Polacheck, R. Li, S.G.M. Uzel and R.D. Kamm, "Microfluidic platforms for mechanobiology", Lab Chip, 13, 2252, 2013.

[4] T. Das, T.K. Maiti and S. Chakraborty, "Augmented stress-responsive characteristics of cell lines in narrow confinements", Int. Biol., 3, 684–695, 2011.

[5] W.T. Jackson, T.H. Giddings Jr, M.P. Taylor, S. Mulinyawe, M. Rabinovitch, R.R. Kopito, K. Kirkegaard, "Subversion of cellular autophagosomal machinery by RNA viruses, Plos. Biol., 3, 5, e156, 2005.

ACKNOWLEDGEMENT

The authors are grateful to Indian Institute of Technology Kharagpur for funding the research.

CONTACT

*Joyjyoti Das, tel: +91-9883010007, jjd.0007@gmail.com