Exploiting the biomimetic properties of multiwall carbon nanotubes in cancer treatment

Mónica L. Fanarraga[1], Juan C. Villegas[2], Lidia Rodriguez-Fernandez[3], Rafael Valiente[4], Jesús Antonio Gonzalez[5]

[1] Departamento de Biología Molecular, Universidad de Cantabria-IFIMAV, 39011, Santander. Spain. fanarrag@unican.es
[2] Departamento de Anatomía y Biología Celular, Universidad de Cantabria-IFIMAV, 39011, Santander. Spain.
[3] SERMET, Universidad de Cantabria, Avda. de Los Castros s/n 39005, Santander, Spain
[4] Departamento de Física Aplicada, Facultad de Ciencias, Universidad de Cantabria, Avda. de Los Castros s/n, 39005 Santander, Spain
[5] MALTA-Consolider Team, CITIMAC, Facultad de Ciencias, Universidad de Cantabria, Avda. de Los Castros s/n, 39005 Santander, Spain jesusantonio.gonzalez@unican.es

Carbon nanotubes (CNTs) have long been blamed for their toxicity leading cell transformation and cancer[1-6]. When examined in detail, these adverse effects are basically the consequence of the interaction of these fibbers with the naturally occurring intracellular nano-filaments, mostly DNA molecules (2 nm diameter) [6,7] and the cytoskeletal polymers, that include actin (6 nm) [8], microtubules (25 nm) [9,10] and intermediate filaments (8-10 nm) [2]. All these intracellular nano-fibbers display different lengths and can form bundles susceptible to interact with CNTs. But microtubules in particular, are highly analogous to CNTs. They are indeed biological nanotubes constituted of 13 protein polymers arranged in a circle that self assembles, display a high resiliency and have a high aspect ratio. Our work unravels the molecular interaction between multiwall CNTs and microtubules in human cancer cells, demonstrating how these nanomaterials can interfere with microtubule function required for cell viability, locomotion and proliferation, leading to cancer cell destruction. These findings could lead to the development of a revolutionary generation of nano-drugs based on the biomimetic properties of multiwall CNTs that could completely transform traditional chemotherapy.

References

[1] Krug, H. F.; Wick, P. Nanotoxicology: An interdisciplinary challenge, Angew. Chem. Int. Ed. Engl. 2011. **50**, 1260-1278
[2] Safi, M.; Yan, M.; Guedeau-Boudeville, M. A.; Conjeaud, H.: Garnier-Thibaud, V.; Boggetto, N.; Baeza-Squiban, A.; Niedergang, F.; Averbeck, D.; Berret, J. F. Interactions between magnetic nanowires and living cells: uptake, toxicity, and degradation. ACS Nano 2011. **5**, 5354-64.
[3] Di Giorgio, M. L.; Di Bucchianico, S.; Ragnelli, A. M.; Aimola, P.; Santucci, S.; Poma, A. Effects of single and multi walled CNTs on macrophages: cyto and genotoxicity and electron microscopy. Mutant. Res. 2011. **722**, 20-31.
[4] Gonzalez, L.; Decordier, I.; Kirsch-Volders, M. Induction of chromosome malsegregation by nanomaterials. Biochem. Soc. Trans. 2010. **38**, 1691-1697.
[5] Sargent, L. M.; Shvedova, A. A.; Hubbs, A. F.; Salisbury, J. L.; Benkovic, S. A.; Kashon, M. L.; Lowry D. T.; Murray, A. R.; Kisin, E. R.; Friend, S.; McKinstry, K. T.; Battelli, L.; Reynolds, S. H. Induction of aneuploidy by single-walled carbon nanotubes. Environ. Mol. Mutagen. 2009. **50**, 708-717.
[6] Cveticanin, J.; Joksic, G.; Leskovac, A.; Petrovic, S.; Sobot, A. V.; Neskovic, O. Using CNTs to induce micronuclei and double strand breaks of the DNA in human cells. Nanotechnology 2010. **21**, 015102.
[7] Li, X.; Peng, Y.; Qu, X. CNTs selective destabilization. of duplex and triplex DNA, inducing B-A transition in solution. Nucleic Acids Res. 2006. **34**, 3670–3676.
[8] Holt, B. D.; Short, P. A.; Rape, A. D.; Wang, Y. L.; Islam, M. F.; Dahl, K. N. Carbon nanotubes reorganize actin structures in cells and ex vivo. ACS Nano 2010. **4**. 4872-4878.
[9] Pampaloni, F.; Florin, E. L. Microtubule architecture: inspiration for novel carbon nanotube-based biomimetic materials. Trends Biotechnol. 2008. **26**. 302-310.
[10] Dinu, C. Z.; Bale, S. S.; Zhu, G.; Dordick, J. S. Tubulin encapsulation of CNTs into functional hybrid assemblies. Small 2009. **5**, 310-315.

Figure 1

HeLa cells incorporate MWCNTs. (left)

TEM of a HeLa cell showing several bundles of MWCNTs (red arrows) within the cytoplasm. **(inset)** A microtubule running in parallel (white arrows) to the bundle is shown. **(rigt)** Raman scattering experiments performed intracellular (red) and extratracellular (black) MWCNT. The Raman spectrum representative for the MWCNT aggregates shows a number of well characterized MWCNT resonances.

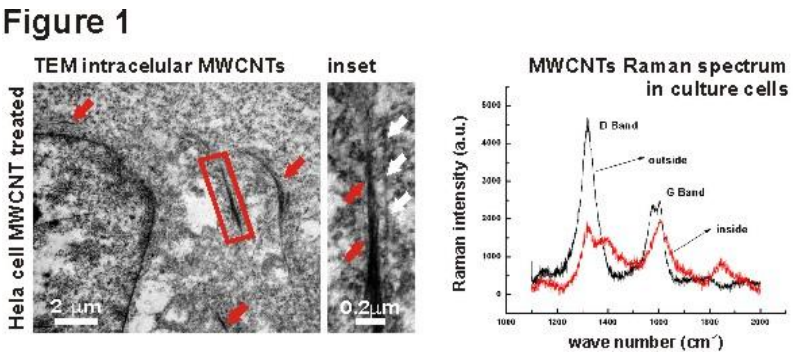

Figure 2

MWCNTs interfere with cancer cell proliferation.

Confocal microscopy image of a HeLa cell undergoing anaphase where an abnormally high density of midzone microtubules is observed (white arrow). This is accompanied by chromosomal bridges (blue arrow) characteristic of clastogenic events (inset, empty arrow). Acentrosomal ectopical microtubule nucleation is observed (red arrows).

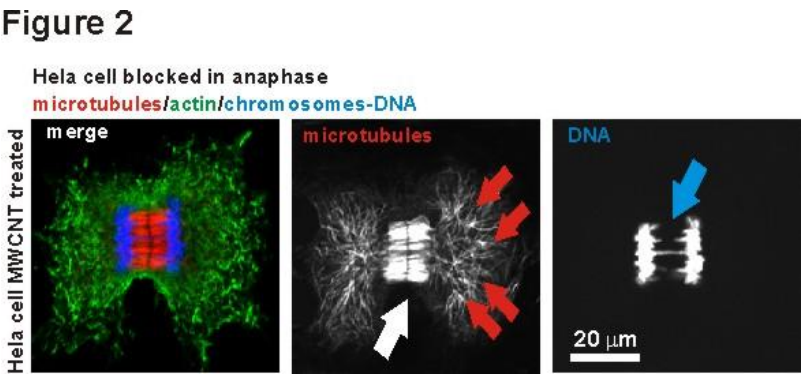

Figure 3

Molecular MWCNT-Microtubule interaction model.

Microtubules are 25 nm diameter nano-tubes build of tubulin. Typically 13 protofilaments associate in a circle to form the microtubule. In contrast to CNTs, microtubules highly continuously undergo assembly/disassembly cycles. The less dynamic end is labeled with the (-) sign, the most dynamic end, indicated with the (+) sign, continuously undergoes rapid assembly/ disassembly cycles. (**botom**) MWCNTs share many structural similarities with microtubules. Inside the cells both types of filaments associate into bundles that increase microtubule resistance to depolymerization, behaving as tubulin scaffolds, and promoting microtubule nucleation and growth