Action of mechanical Cues in vivo on the Growth of a subcutaneously grafted Tumor: Proof of Concept

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Abstract

The cancerous tumor tissue and its extracellular matrix are subject to mechanical signals. The role of pressure in tumor transformation and growth as well as in the appearance of metastasis is more and more understood. Hence the effect of constraint/stress on tumor growth has been widely explored in vitro in 3-dimension cell culture. The proof of concept delivered by the present work shows the effect of a constraint field in vivo on tumor growth. Nude mice were grafted subcutaneously with a mix of ferric nanoparticles and MDA MB 231 cells. The nanoparticles with a diameter of 100 nm rapidly spread around the growing tumor. The field of constraint was applied through the magnetized nanoparticles located around the tumor. It was generated by the action of a magnetic field gradient on the nanoparticles using permanent magnets located outside the animal. A very statistically significant difference (p=0.015) was observed between the volume of tumors with nanoparticles around and subjected to a field of constraint for 2 hours/day for 21 days and observed to day 59 or more, and the volume of tumor of the three control groups. This experiment provides the first evidence of an action of mechanical signals on the growth of tumor in vivo, in animal. These results confirm in vivo the results previously obtained in vitro on 3-dimension tissue culture models.

References

1. Cross SE, *et al.* (2008) AFM-based analysis of human metastatic cancer cells. *Nanotechnology* 19(38):384003.
2. Murphy MF, *et al.* (2013) Evaluation of a nonlinear Hertzian-based model reveals prostate cancer cells respond differently to force than normal prostate cells. *Microsc Res Tech* 76(1):36-41.
3. Remmerbach TW, *et al.* (2009) Oral cancer diagnosis by mechanical phenotyping. *Cancer Res* 69(5):1728-1732.
4. Fuhrmann A, *et al.* (2011) AFM stiffness nanotomography of normal, metaplastic and dysplastic human esophageal cells. *Phys Biol* 8(1):015007.
5. Canetta E, *et al.* (2014) Discrimination of bladder cancer cells from normal urothelial cells with high specificity and sensitivity: combined application of atomic force microscopy and modulated Raman spectroscopy. *Acta Biomater* 10(5):2043-2055.
6. Indra I (2012) Mechanical forces and tumor cells: insight into the biophysical aspects of cancer progression. *Wayne State University Dissertations.* http://digitalcommons.wayne.edu/oa_dissertations/413:Paper 413.
7. Xu W, *et al.* (2012) Cell stiffness is a biomarker of the metastatic potential of ovarian cancer cells. *PLoS One* 7(10):e46609.
8. Plodinec M, *et al.* (2012) The nanomechanical signature of breast cancer. *Nat Nanotechnol* 7(11):757-765.
9. Lekka M, *et al.* (2012) Cancer cell recognition--mechanical phenotype. *Micron* 43(12):1259-1266.
10. Ingber DE, Madri JA, & Jamieson JD (1981) Role of basal lamina in neoplastic disorganization of tissue architecture. *Proc Natl Acad Sci U S A* 78(6):3901-3905.
11. Tse JM, *et al.* (2012) Mechanical compression drives cancer cells toward invasive phenotype. *Proc Natl Acad Sci U S A* 109(3):911-916.
12. Lee GY, Kenny PA, Lee EH, & Bissell MJ (2007) Three-dimensional culture models of normal and malignant breast epithelial cells. *Nat Methods* 4(4):359-365.
13. Jonietz E (2012) Mechanics: The forces of cancer. *Nature* 491(7425):S56-57.
14. Baish JW & Jain RK (2000) Fractals and cancer. *Cancer Res* 60(14):3683-3688.
15. Bizzarri M, *et al.* (2011) Fractal analysis in a systems biology approach to cancer. *Semin Cancer Biol* 21(3):175-182.
16. D'Anselmi F, *et al.* (2011) Metabolism and cell shape in cancer: a fractal analysis. *Int J Biochem Cell Biol* 43(7):1052-1058.
17. Stein GS, *et al.* (1999) Implications for interrelationships between nuclear architecture and control of gene expression under microgravity conditions. *FASEB J* 13 Suppl:S157-166.
18. Xu R, Boudreau A, & Bissell MJ (2009) Tissue architecture and function: dynamic reciprocity via extra- and intra-cellular matrices. *Cancer Metastasis Rev* 28(1-2):167-176.
19. Ingber DE (2006) Cellular mechanotransduction: putting all the pieces together again. *FASEB J* 20(7):811-827.
20. Paszek MJ, *et al.* (2005) Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8(3):241-254.
21. Montel F, *et al.* (2011) Stress clamp experiments on multicellular tumor spheroids. *Phys Rev Lett* 107(18):188102.

Figures

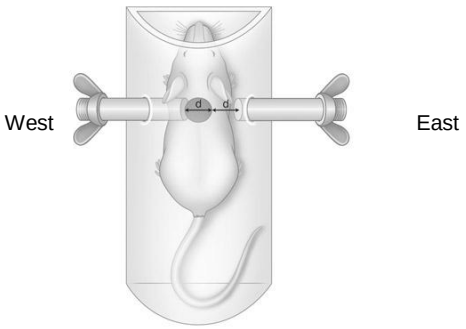


Fig. 1- A: Schematic representation of the experimental setup with the animal (Magnets tumor not at scale)

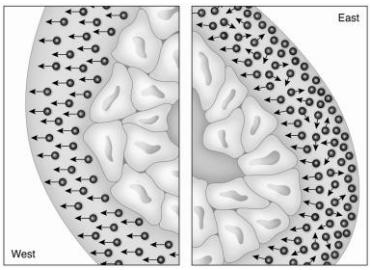
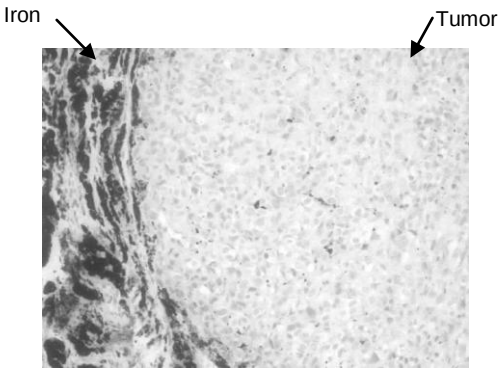


Fig. 1- B: Close up of the West and East sides with force vector



Tumor MDA MB 231, Perls special stain, x100-Important labeling of peri-tumoral areas

Fig. 2 - Spreading of the nanoparticles around a subcutaneous grafted tumor

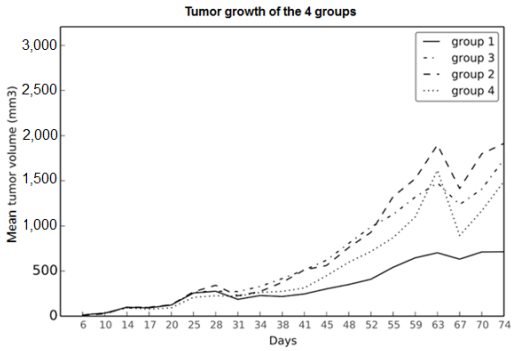


Fig. 3 - Growth curve of the tumors in the 4 groups

Volume (mm^3)	Median(Q1; Q3)	(Min; Max)	Mean (±std)	Significance (p value)
Treated (N=7)	529 (502; 840)	346; 966	646±235	Significant **(p=0.015)**
Controls (N=33)	1,334 (758; 1784)	256; 2106	1,250±282	IC 95% 579 (124; 1,099)

Table 1 - Tumor volume measured on D59+tumors