CDK Inhibitor PD 0332991 Selectively Inhibits Lung Adenocarcinoma Cells Without Sacrificing Matrix Embedded Endothelial Cells Ability Regulatory Effect on Tumor Proliferation

Gabriel L. A. Cunha[1,2], Alina Freiman[1], Laura Indolfi[1], Natalie Artzi[1], Juliana L. Dreyfuss,[1,2,3] and Elazer R. Edelman[1,4].

[1]Biomedical Engineering Center, Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA. [2]Department of Biochemistry, [3]Department of Health Informatics, Interdisciplinary Sciences in Health Technology Group, Universidade Federal de São Paulo, São Paulo, Brazil. [4]Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA.

Statement of Purpose: Our group has recently shown that endothelial cells (ECs) play a significant role in tumor proliferation and invasiveness by paracrine regulation (Franses, 2011). Embedding of endothelial cells in 3D matrices of denatured collagen (MEECs) are capable of blocking tumor growth of lung and breast carcinomas *in vitro* and *in vivo* (unpublished results). Covalent conjugation of heparin to the matrix surface enhanced the anti-proliferative effect of embedded EC and elevated secretion of heparan sulfate (unpublished results). We hypothesized that the surface modification with heparin provided a reservoir potential for matrices that could enable positively charged drugs to bind and be slowly released – augmenting cellular effects. We examined the biologic effects of PD 0332991 and MEEC. This positively charged selective inhibitor of CDK4/cyclin D1 and CDK6/cyclin D2 (Fry, 2004) is reported to inhibit tumor growth with little effect on adjacent cells (Choi, 2012; Fry, 2004; Finn, 2009). PD 0332991 successfully inhibited tumor cells without affecting EC and shows promise as a combined therapy with heparin-MEECs targeting lung carcinoma cells.

Methods: Primary human umbilical vein endothelial cells (ECs; Lonza) and A549 lung carcinoma cells (ATCC) were cultured under standard conditions. PD 0332991 HCl (PD-HCl) and PD 0332991 isethionate (PD-ISE) were from Selleck and Sigma. Cell viability was assessed through resazurin reduction. 10^4 cells/well were seeded in 96-well plates (Corning) and incubated overnight (37 ºC; 5% CO_2) and then treated with 1, 10 and 100 μM of PD-ISE or PD-HCl in serum free medium. On the next day 10% (v/v) of 440 μM resazurin was added to the wells. Fluorescence (ex560/em590) was measured after 24, 48 and 72 hours of incubation. Data were analyzed using One-way ANOVA followed by Dunnet's post-test. Viability of treated cells was expressed as percentage of viable cells compared to untreated controls.

Results: Dose and temporal response to PD 0332991 (either PD-HCl or PD-ISE) was examined to find the optimal conditions for blocking cancer cell proliferation without harming EC. While 1 μM of both compounds had no effect on EC or cancer cells, 24 hour incubation with 100 μM solutions affected cancer and endothelial cells viability nonspecifically (30 and 13% viable cells, respectively). 10 μM solutions acted only on cancer cells with visible effects after 24 hours (Fig.1). 10 μM PDE-ISE was significantly more efficient in cancer cell inhibition compared with PD-HCl (≈ 50% vs. 85% viable cells after 72 h). PDE-ISE was selectively toxic towards A549 cells and not ECs (≈ 50% vs. 97% viable cells at 72 h) (Fig. 2).

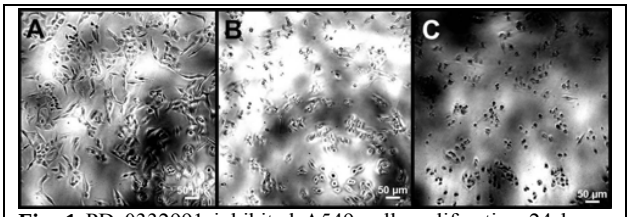

Fig. 1 PD 0332991 inhibited A549 cell proliferation 24 hours post incubation. A: untreated; B: PD-HCl, C: PD-ISE. (100x)

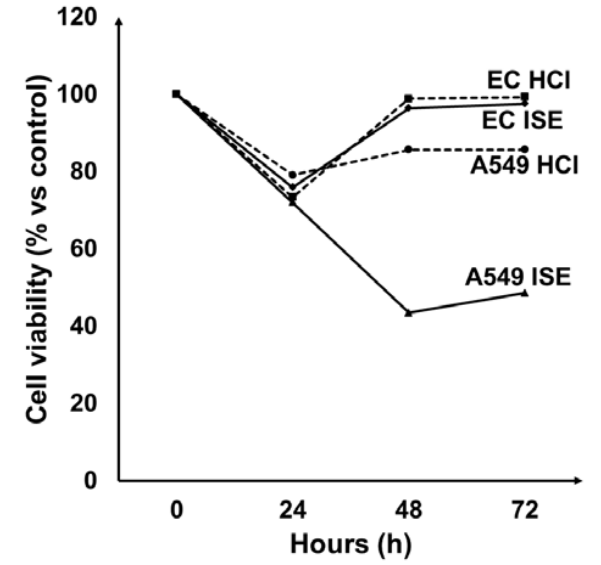

Fig.2 Viability of ECs and A549 cells are affected by PD0332991 but while EC recover A549 are permanently inhibited for the ISE formulation more than the HCl.

Conclusions: Surface modification of matrices used for tissue engineering enables concomitant release of drugs from cell bioreactors. Release of CyclinD1/D2 pharmacologic blocker selectively target cancer cells while maintaining EC viability, making it a suitable drug to combine with heparin-MEEC implants cell therapy.

References:

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