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Integrin adjunct therapy for melanoma

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DEAR EDITOR,

We observed that in human M21 melanoma cells, chemotherapy was effective with adherent cells yet suspended cells were highly resistant [1]. Moreover, in 4/5 randomly chosen primary melanoma isolates, cell detachment induced chemoresistance. These results are of particular relevance because melanomas typically grow in densely packed cellular “nests” that contain minimal extracellular matrix [2]. M21 cells grown as subcutaneous xenografts in SCID mice also lack detectable fibronectin, collagen or laminin, indicating loss of extracellular matrix [1], similar to human tumor specimens. Thus, integrin engagement and signaling may be low even in solid tumors. Co-administration of reagents that stimulated integrin signaling to tumor-bearing mice potentially enhanced melanoma responses to chemotherapy [1]. These results strongly supported the idea that loss of integrin signaling contributes to therapy resistance *in vivo*. However, the reagents used in that study (the snake venom disintegrin contortrostatin and the activating integrin $\beta 1$ antibody TS2/16) are anticipated to have side effects, thus, are unlikely to be suitable for use in humans.

Numerous studies have shown that clustering is the key event in integrin signaling [3]. Multivalent integrin-binding reagents can cluster integrins to mimic adhesion to extracellular matrices [4]. Multivalency also increases affinity for cells so that reagents are effective at low concentrations [5–7].

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Disclosure of Potential Conflict of Interest

No potential conflicts of interest

We therefore constructed a pentavalent fibronectin (FN-COMP; Figure 1A and data S1) in which the integrin binding region (type III repeats 5–11) was linked to the short cartilage oligomeric peptide (COMP) that mediates pentamer formation [7]. SDS-PAGE of the purified proteins under reducing and non-reducing conditions gave rise to major bands of the expected molecular mass (Figure 1B). Gel filtration chromatography confirmed that the protein formed a large complex that ran as a single species, demonstrating efficient oligomerization (Figure 1C). Both the monomer and the pentamer migrate at positions that are larger than their expected molecular masses, which could be due to an elongated conformation or to additional protein-protein interactions, as previously suggested [7].

FN-COMP contains the region of fibronectin that binds to integrins $\alpha 5\beta 1$ and $\alpha V\beta 3$, both of which are commonly expressed on melanomas [8]. A distinct fibronectin pentamer was previously found to bind and localize with $\alpha 5\beta 1$ integrin [7]. Next we tested the ability of this construct to stimulate integrin signaling. Addition of this protein to suspended cells triggered increased tyrosine phosphorylation of bands around 60, 75 and 125 kD (Figure 1D and S2), which are characteristic of integrin signaling [9]. Furthermore, we saw increase >250 percent increase in FAK phosphorylation 30 min after treatment when compared to untreated control (Figure 1D).

We next tested whether adding FN-COMP would enhance the response to therapy in non-adherent human melanoma cells. These experiments used M21 and VMM12 cells, two melanoma lines that show decreased chemosensitivity after detachment [1]. We found that addition of FN-COMP to non-adherent melanoma cells improved chemo and radiosensitivity (Figure 2A and 2B). By contrast, FN-COMP showed no effect on VMM18 cells, a line where chemosensitivity is independent of cell adhesion (Figure S3). FN-COMP is therefore able to enhance therapy sensitivity of melanoma cells that show detachment-induced chemoresistance. Since relatively modest differences in chemosensitivity *in vitro* can have major effect on patient survival [10], these data support the idea that restoring therapy sensitivity in non-adherent melanomas may be clinically relevant.

We first assessed the ability of FN-COMP to enter the blood and its stability after intraperitoneal injection into mice. Initial studies showed that it appeared in the blood by 3h and was stable for at least 24h after injection (Figure S4). Next, mice bearing subcutaneous M21 tumors were treated with cisplatin alone, FN-COMP alone, or cisplatin plus FN-COMP, receiving three treatments ~1 week apart. Tumor growth was then monitored (Figure 2C). Neither FN-COMP alone nor cisplatin alone had significant effects on tumor growth (FN-COMP *vs.* control: $P=0.177$; cisplatin *vs.* control: $P=0.432$). The combined treatment, however, dramatically decreased tumor size. When compared with sum of the individual effects of cisplatin and FN-COMP, the combined treatment was clearly synergistic (cisplatin *vs.* FN-COMP+cisplatin: $P=0.0031$). Remarkably, dramatic results were observed after only 3 treatments, compared with regimens of many months for chemotherapy in patients. Not unexpectedly, tumors resumed growth ~5 weeks after treatment ended. However, the substantial effects on an otherwise therapy-resistant line with this limited treatment regimen are impressive and suggest that stronger responses may be obtained using longer protocols. Side effects in the mice were not observed. These data therefore strongly argue that loss of

integrin-mediated adhesion is an important causative factor in therapy resistance of melanoma and that FN-COMP can reverse this effect.

In summary, combining chemotherapy or radiotherapy with FN-COMP to stimulate integrin signaling, offers a new direction for improving therapeutic outcomes in the treatment of otherwise intractable metastatic melanomas. Further work to test FN-COMP efficacy and specificity in clinical cancer therapy seems indicated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure-1.

Characterization of FN-COMP. Schematic representation of FN-COMP. Numbered ovals denote fibronectin type-III repeats (5–11); black rectangle denotes the assembly domain of COMP. C-terminal His6 not shown. (B) Coomassie-stained SDS-PAGE gel. FN-COMP was run under reduced (R) and non-reduced (NR) conditions. (C) Gel filtration chromatography of FN-COMP and fibronectin monomer (FN) as described under Materials and Methods. Standards are: Blue dextran, excluded volume; thyroglobulin, 669,000; catalase, 232,000; ferritin, 440,000; BSA, 67,000 Da. (D) 3T3 cells were left adherent (lane 1) or detached and kept in suspension (lane 2: control) for 1h. Suspended cells were either treated with of FN-COMP at 40µg/ml for 15 (lane 3) or 30 minutes (lane 4) or left untreated. Lysates were analyzed by Western blotting using anti-phosphotyrosine antibody pY20. Tubulin was used as a loading control. n=2. (E) 3T3 cells were left adherent (lane 4) or detached and kept in suspension (lane 1) for 1h. Suspended cells were either treated with of FN-COMP at 40µg/ml for 15 (lane 3) or 30 minutes (lane 4) or left untreated (lane 1: control). Lysates were analyzed by Western blotting using FAKpy397 antibody pY20 and total FAK. Tubulin was used as a loading control. Bar graph shows quantification of western blot. n=2.

Figure-2.

FN-COMP in chemotherapy *in vitro* and *vivo*. (A) M21 and VMM18 melanoma cells were left adherent (Ad) or detached and kept in suspension (Sus) for 1h. Suspended cells were then treated with of FN-COMP (FNC) at 40µg/ml for 30–45 min followed by treatment with either 10Gy X-irradiation or cisplatin at 4µM. Cell survival was assayed 72h later. Values are means $\pm$ SE, n=3. *P<0.05. (C) Mice with palpable subcutaneous tumors were treated with cisplatin (CD), FN-COMP (FNC) alone, or CD plus FNC. Mice received 3 successive treatments 1 week apart. Tumor volumes were then followed. Values are means $\pm$ SE, n=10–

15 mice. For CD vs. CD+FNC: * $P < 0.0031$. The error bars are asymmetric because the analysis was done in the log-scale, and then converted back to the original scale. Qualitatively similar results were obtained in 2 independent experiments.

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