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Characterization of EGF-guided MDA-MB-231 cell chemotaxis *in vitro* using a physiological and highly sensitive assay system

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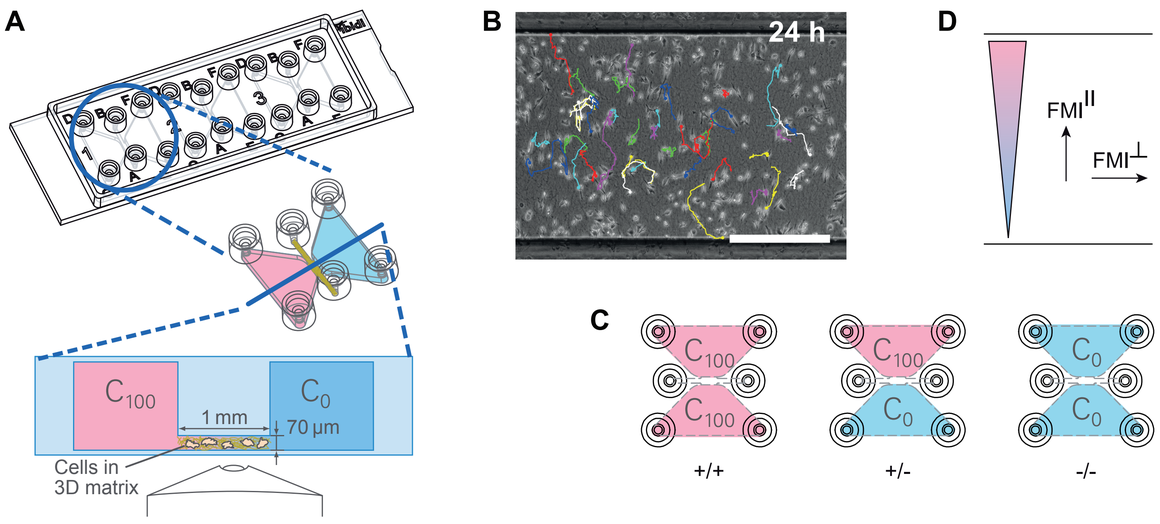
Abstract

Chemotactic cell migration is a central mechanism during cancer cell invasion and hence metastasis. In order to mimic *in vivo* conditions we used a three dimensional hydrogel matrix made of collagen I and a stable gradient-generating chemotaxis assay system, which is commercially available (µ-Slide Chemotaxis), to characterize epidermal growth factor (EGF)-induced chemotaxis of the human breast cancer cell line MDA-MB-231.

Surprisingly, chemotactic effects of EGF on MDA-MB-231 cells could neither be observed in the standard growth medium DMEM/F-12 supplemented with 10 % serum nor in starvation medium. However, after adapting the cells to the serum-free growth medium UltraCULTURE™, significant chemotactic effects could be measured with high sensitivity. The extremely time-stable linear gradients, generated in the chemotaxis chamber, led to consistent dose dependent directional migration of MDA-MB-231 cells towards stable gradients of EGF. Both, blocking the ligand-binding domain of the EGF receptor (EGFR) by an antibody (monoclonal anti-EGFR antibody 225) and inhibition of its kinase domain by a small molecule inhibitor (AG1478) led to a reduction in EGF-induced directed migration, confirming EGF as potent chemotactic guidance cue for MDA-MB-231 migration. Additionally, the high sensitivity of the assay even allowed us to observe synergistic effects in EGFR inhibition using a combination of low doses of both inhibitor types.

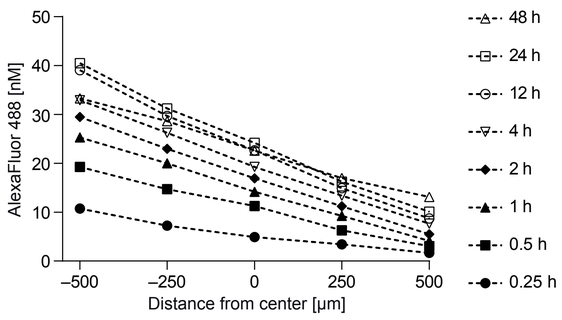
Physiological 3D chemotaxis and migration assay

Experimental setup and data analysis



(A) Scheme of the µ-Slide Chemotaxis. (B) MDA-MB-231 cancer cells migrating in a collagen I matrix within the observation area of a chemotaxis chamber. (C and D) Internal controls increase sensitivity of the analysis. (C) Experimental condition and functional controls on the same assay slide increase analysis sensitivity. (D) Analysis of cell directionality (Forward Migration Indices, FMIs) in gradient direction (FMI^{II}) and perpendicular (FMI$^{\perp}$) to the gradient to identify and validate biased migration.

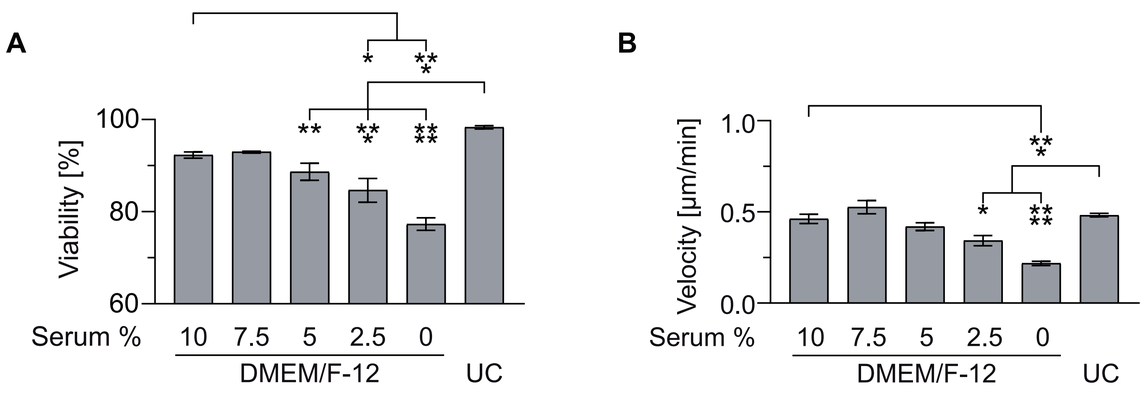
Highly stable gradients allow the analysis of chemotactic responses of slowly migrating cancer cells



Within two hours after filling of the reservoirs, stable linear gradients were generated in the collagen matrix of the migration chamber. Gradients remained constant for at least 48h. Time series were obtained by fluorescence correlation spectroscopy of AlexaFluor 488 gradients.

Serum-free growth medium preserves cell fitness

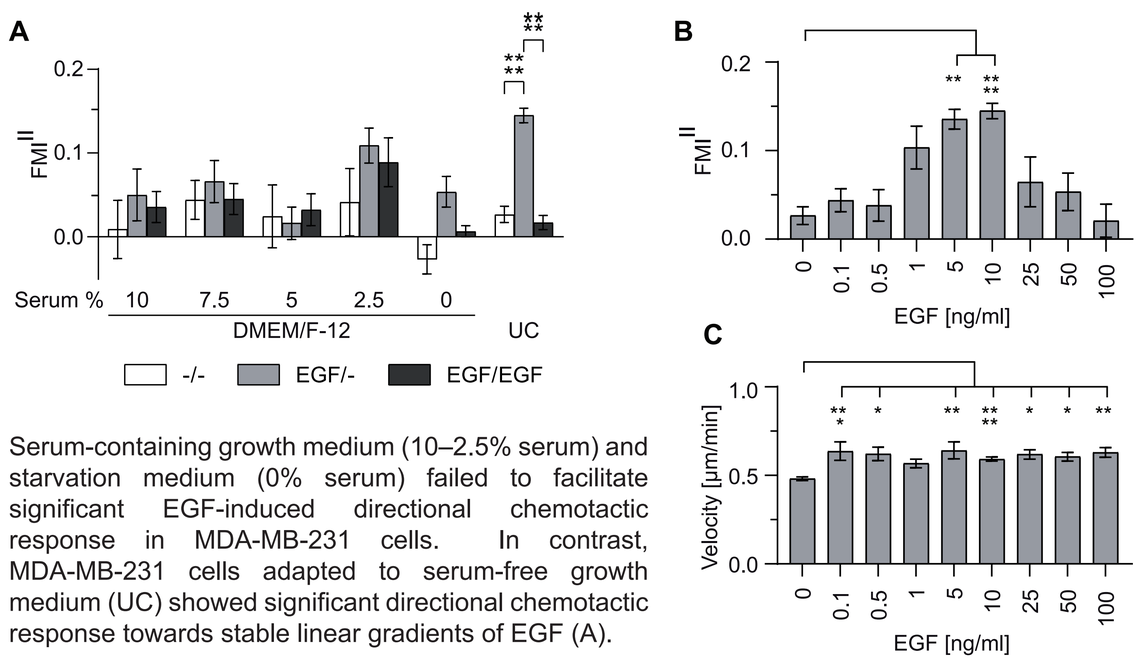
Serum-free growth medium preserves cell fitness without negative effects of serum starvation



MDA-MB-231 cells adapted to serum-free growth medium UltraCULTURE™ (UC) showed cell viability (A) and migration velocity (B) comparable to MDA-MB-231 cells in growth medium supplemented with 10% bovine serum. Reduction of the serum content in standard growth medium (7.5–2.5% serum) or starvation medium (0% serum), however, had a negative effect on cell vitality and migration velocity.

MDA-MB-231 guidance by stable gradients of EGF

Serum-free growth medium enhances MDA-MB-231 sensitivity and allows the characterization of EGF-guided chemotaxis



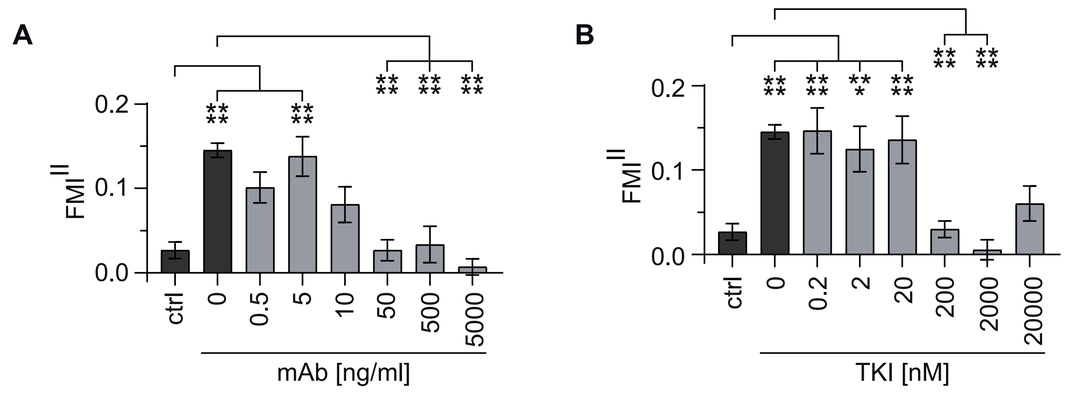
Serum-containing growth medium (10–2.5% serum) and starvation medium (0% serum) failed to facilitate significant EGF-induced directional chemotactic response in MDA-MB-231 cells. In contrast, MDA-MB-231 cells adapted to serum-free growth medium (UC) showed significant directional chemotactic response towards stable linear gradients of EGF (A).

EGF-induced directional, but not kinetic response is dosage-dependent

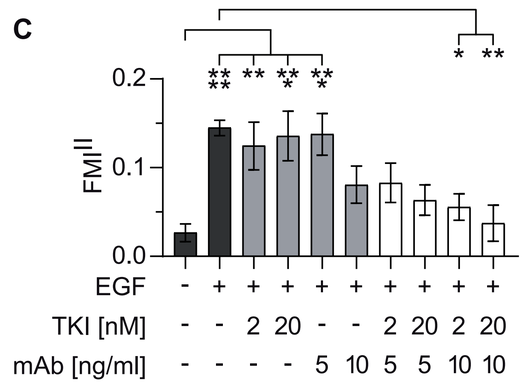
The observed directional responses of MDA-MB-231 cells were maximal at EGF concentrations around 10 ng/mL (B). Already low concentrations of EGF were sufficient to induce significant kinetic responses (C) However, no dosage dependent effect could be observed. Those results imply a global receptor saturation at EGF concentrations higher than 10 ng/mL.

Synergistic EGFR inhibition at low inhibitor doses

Global inhibition (mAb) and tyrosine kinase domain inhibition (TKI) of EGFR reduces chemotactic responses in a dosage dependent manner



Effective synergistic inhibition of EGFR by combination of low doses of different inhibitor types



Both, inhibition of the EGFR ligand binding domain by a blocking antibody (mAb, A) and the tyrosine kinase domain by a small molecule inhibitor (TKI, B) led to dose-dependent reduction of the chemotactic response of MDA-MB-231 cells. Combining both inhibition strategies allowed us to significantly reduce the effective concentration of the respective inhibitor (C).