xCELLigence System Real-Time Cell Analyzer

Focus Application
Cell Migration

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Featured Study: Inhibition of Cell Migration by Gene Silencing

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Introduction

Cell migration is an important process in the development of organisms (1), as well as during immune surveillance and wound healing. The process of directed cell migration includes the recognition of extracellular signals or attractants, as well as coordinated cooperation between the cellular polarity machinery, integrin trafficking, and intracellular signaling (2). For cancer development, the potential of cells to migrate or to grow invasively is, in addition to proliferation, the most important disease-causing factor. The inhibition of cell migration is a key target of the new anti-inflammatory therapies (3).

In this study, we investigated the influence of cellular protein X on the migration potential of a wide range of tumor entities represented by diverse cell lines. Our data present evidence that protein X is involved in the general migration pathway in all cancers examined, providing a possible new therapeutic target. These findings are starting points for further investigations to understand the cellular processes involved in migration at the molecular level.

Materials and Methods

Cell culture: PC3, T24, HT29, MCF7, TX3868, and HT1080 cells were cultured in DMEM medium (Gibco), containing 10% FBS (Biochrom) and 1% penicillin/streptomycin (PAA). H1299 cells were cultured in RPMI 1640 medium (PAA) containing the same supplements. All cells were incubated in a humidified environment at +37°C with 5% CO_2 during cultivation, siRNA treatment, and migration assays.

siRNA-dependent silencing of gene expression: For gene silencing, 1.2×10^5 cells/well were seeded in 12-well plates and 2.4×10^5 cells/well in 6-well plates. Cells were transfected with specific siRNA directed against cellular mRNA for protein X or control siRNA (AllStars Negative Control siRNA, Qiagen) using HiPerFect Transfection Reagent (Qiagen), following the manufacturer's instructions. After 24 hours, medium was changed and cells were transfected a second time.

Cell migration assays: Cell migration was tested in the BD Falcon FluoroBlok system (BD Biosystems) in 24-well inserts. Approximately 2.5×10^4 PC3 cells treated with control siRNA or specific siRNA X were seeded in DMEM medium containing 0.5% FBS. Inserts were placed into DMEM medium with 10% FBS as an attractant. After 24, 48, and 72 hours, cells were fixed with methanol, stained with propidium iodide or DAPI, and analyzed by fluorescence microscopy.

The findings of this approach were compared to the new technique of real-time migration monitoring using the CIM-Plate 16 and xCELLigence System RTCA DP Instrument (ACEA). In this system, 1.0×10^4 and 2.0×10^4 cells treated either with siRNA (specific or control) or left untreated, were seeded in the upper chamber of a CIM-Plate 16 in the above described medium of each respective cell line without FBS. The upper chamber was then placed on the lower chamber of the CIM-Plate 16 containing growth medium supplemented with 10% FBS as an attractant, or without FBS (negative control). Cell migration was monitored over a period of up to 18 hours.

Results

In our first approach, we tested the migration potential of several tumor cell lines in a trans-well migration system. Migration was stopped after 24, 48, or 72 hours by fixing the cells with methanol; cells were then stained with propidium iodide. HT1080 and H1299 cells were analyzed after only 24 hours because the optimal time point for these cell lines had been identified in previous studies. After 24 hours, HT1080, T24, and TX3868 cells showed a high migration potential indicated by the presence of numerous stained cells on the lower side of the membrane. The number of stained cells was even higher for T24 and TX3868 cells after 48 or 72 hours (see Figure 1, cell lines 1-3). For PC3 cells, we found an intermediate migration potential indicated by a lower number of stained cells after 24 hours; the number of stained cells rose over 48 to 72 hours (see Figure 1, cell line 5). Low or no migration was found for the remaining cell lines, HT29 and MCF7 (see Figure 1, cell lines 6-7).

To confirm the results and to obtain more information about the migration kinetics, we tested the same cell lines using the RTCA DP Instrument with the CIM-Plate 16. In the xCELLigence System, TX3868, H1299, and T24 cells again showed the fastest migration. Within the first 6 hours, HT1080 cells showed migration kinetics similar to that of H1299 cells. In keeping with the results of the endpoint measurement, PC3 cells showed intermediate migration kinetics, while HT29 and MCF7 cells showed no migration and were comparable to the negative control (see Figure 2). Real-time monitoring of cell migration allowed much faster collection of results compared to the endpoint measurement, providing comparable results by 18 hours instead of 72 hours cell culture. In addition, real-time monitoring of cell migration provided a clearer more precise kinetics analysis, enabling discrimination between migration and later proliferation effects. In contrast, strict reliance on the endpoint analysis would not have achieved this result.

In order to establish optimal conditions, we selected the T24 cell line, showing high migration potential using both endpoint assays and real-time cell analysis. We tested if the specific cancer-associated protein X is necessary for cell migration. To answer this question, we reduced the cellular level of protein X in T24 cells by transfection of a specific

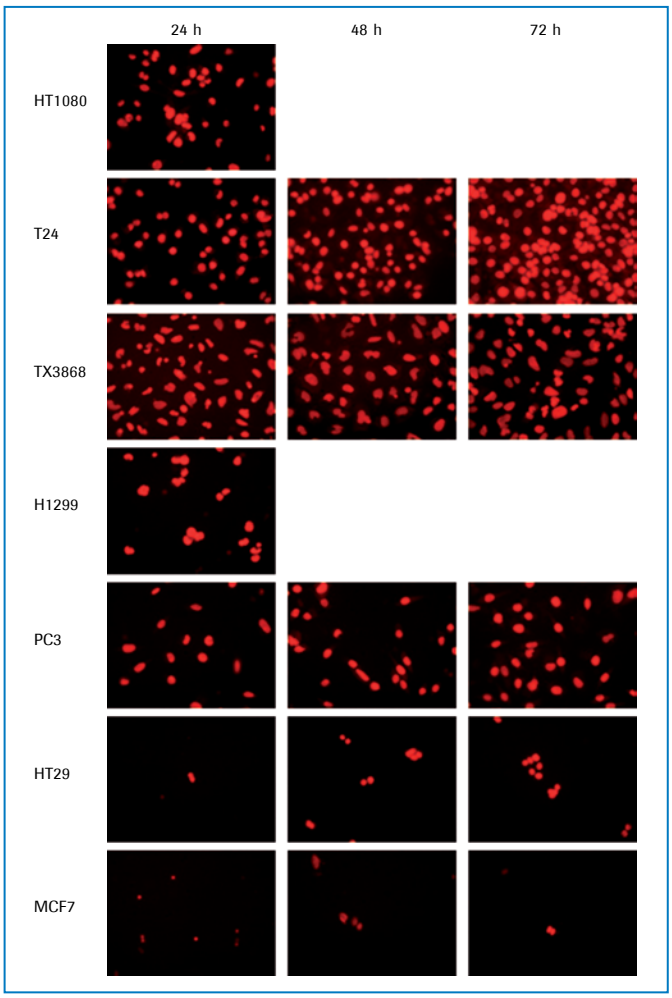

Figure 1: Microscopic analysis of migrated cells after staining. Methanol-fixed and propidium iodide-stained cells 24, 48, or 72 hours after seeding in the BD Falcon FluoroBlok system in 24-well inserts. A FBS gradient from 0.5% to 10% was used as an attractant.

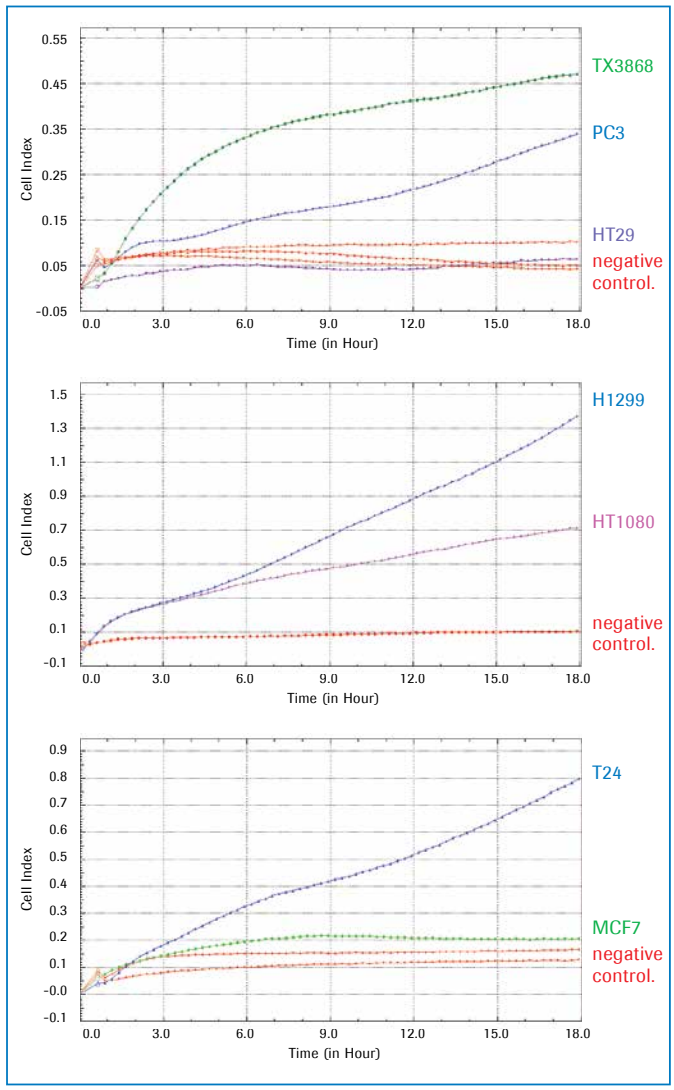

Figure 2: Real-time monitoring of cell migration using the xCELLigence System in combination with the CIM-Plate 16. Approximately 1.0 x 10^4 cells were seeded into wells of a CIM-Plate 16, and the migration behaviour of each of the cell lines was monitored for 18 hours.

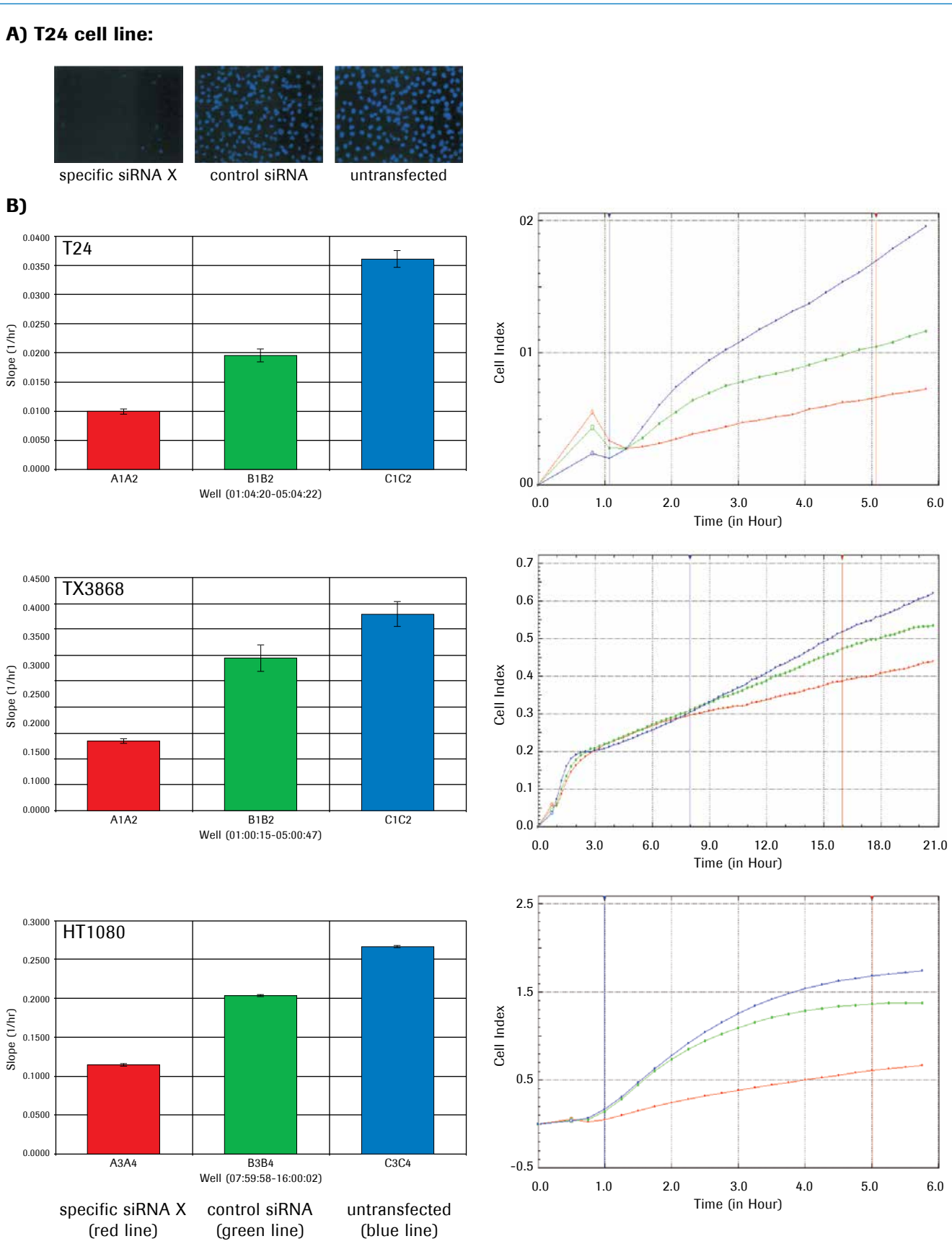

Figure 3: A) Cell migration detection after gene silencing. Approximately 2.5 × 10^4 T24 cells (either untransfected, transfected with control siRNA or specific siRNA X) were seeded in the BD Falcon FluoroBlok system (BD Biosystems) in 24-well inserts. A FBS gradient from 0.5% to 10% was used as an attractant. The pictures show methanol-fixed and DAPI-stained cells after 48 hours of migration.
B) Cells were transfected in the same way and 1.0 × 10^4 cells were seeded in CIM-Plates 16 (ACEA) 72 hours after first transfection. Using the xCELLigence RTCA DP Instrument (ACEA), migration curves of T24, TX3868, and HT1080 cells were monitored online (right panels), and the slope of the migration curve was calculated using the RTCA 1.2 Software (left panel).

siRNA X directed against the respective mRNA. Again, we compared real-time migration monitoring with the results of DAPI-stained cells after migration in the BD Falcon FluoroBlok system, which served as our endpoint method (shown for T24 cells, see Figure 3a).
In Figure 3b, bars represent the slope of the migration curves calculated using RTCA Software 1.2 for T24, TX3868, and HT1080 cells assayed in CIM-Plates 16. For all cell lines tested, we observed a significant reduction in migration after the silencing of protein X using siRNA. The effect was even more pronounced in the HT1080 and TX3868 cells, because T24 cells appeared to be more affected by the transfection procedure itself. Only real-time cell monitoring enabled the identification of the reduced migration in T24 cells after transfection with control siRNA. Our data show that protein X is required for cancer cell migration.

Conclusion

Performing real-time monitoring of cell migration instead of endpoint measurement provided significant advantages. The new technique made possible by the xCELLigence RTCA DP Instrument using CIM-Plate 16 is much faster and less laborious. In addition, time point optimization experiments are no longer necessary as migration is monitored online. Finally, the ability to combine migration monitoring with proliferation measurements using both an E-Plate 16 to measure cell proliferation and a CIM-Plate 16 to quantify migration in a single experiment, serves as a direct control showing that reduced migration is not an effect of reduced cell viability.

References

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Key words:
cancer, gene silencing, migration, xCELLigence System, RTCA DP Instrument, real-time migration monitoring, CIM-Plate 16

Ordering Information

Product	Cat. No.	Pack Size
xCELLigence RTCA DP Instrument	**00380601050**	**1 Bundled Package**
RTCA DP Analyzer	05469759001	1 Instrument
RTCA Control Unit	05454417001	1 Notebook PC
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E-Plate VIEW 16	06324738001	6 Plates
	06324746001	6 x 6 Plates
E-Plate Insert 16	06465382001	1 x 6 Devices (6 16-Well Inserts)
CIM-Plate 16	05665817001	6 Plates
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