

CDK4/6 kinase inhibitor Palbociclib influences migration of different malign and non-malign cell lines independent of irradiation

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1. Background

Since 2015 the kinase inhibitor Palbociclib (Ibrance) is approved as a treatment for HR⁺, HER2⁻ advanced or metastatic breast cancer. More than 79,000 patients were treated since then. Palbociclib binds selectively to the cell cycle proteins CDK4 and CDK6 and arrests the cells in G1 phase. The need for an additional radiotherapeutic treatment could appear, when a patient develops metastasis despite a kinase inhibitor therapy. Then the question is whether irradiation treatment does effects the migration in an advanced or metastatic situation? To benefit from a migration influencing effect of Palbociclib, an earlier treatment in contrast to the advanced or metastatic stage could be more efficient for the patients. To address these questions we studied the influence of the inhibitor and a supplementary irradiation on the migration behavior in a scratch assay setting.

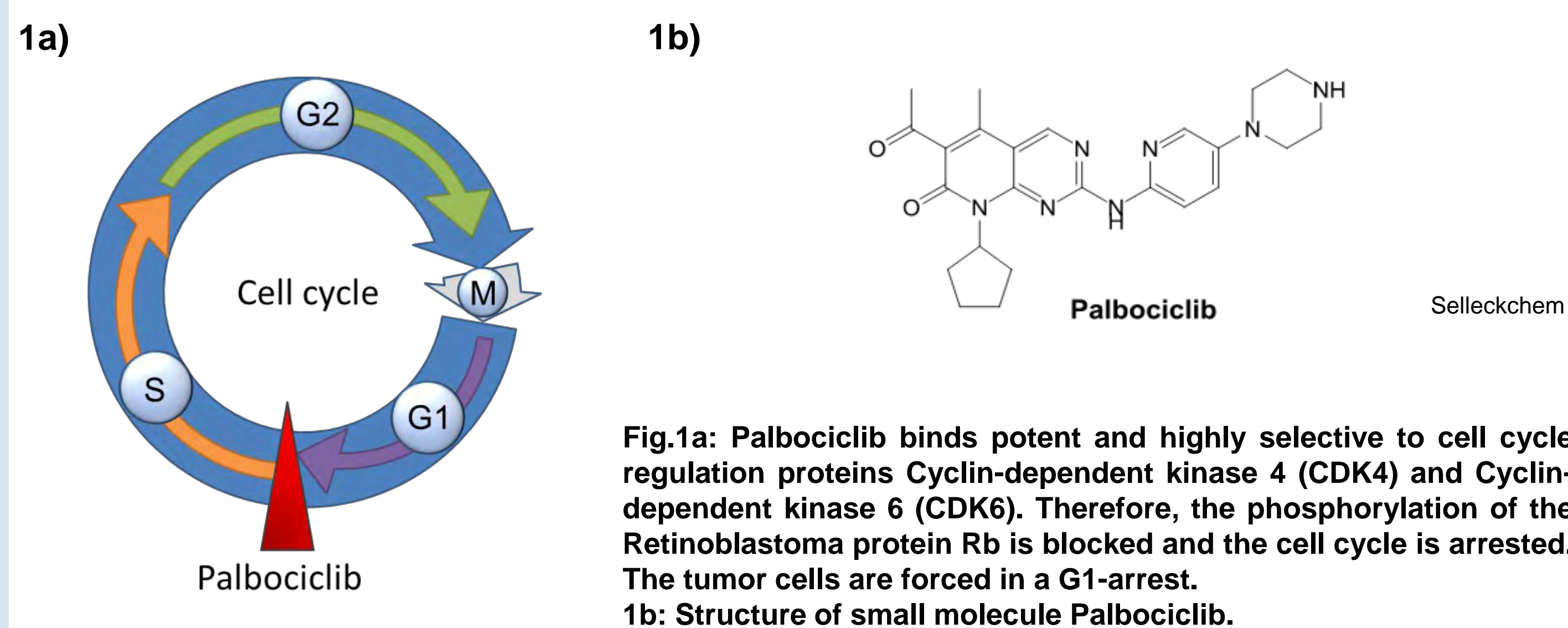


Fig.1a: Palbociclib binds potent and highly selective to cell cycle regulation proteins Cyclin-dependent kinase 4 (CDK4) and Cyclin-dependent kinase 6 (CDK6). Therefore, the phosphorylation of the Retinoblastoma protein Rb is blocked and the cell cycle is arrested. The tumor cells are forced in a G1-arrest.

1b): Structure of small molecule Palbociclib.

2. Methods

Different primary cell cultures from melanoma patients, as well as breast carcinoma cell lines were used to perform scratch assays. As a control fibroblasts of a healthy donor were used too. Cells were seeded in 48-well-plates and starved for 24 h in minimal-medium (2 % FCS). Confluent cells were scratched with a 10 µL-pipet tip. Wells were treated either with inhibitor, ionizing radiation or a combination of both treatments. Images were taken with 40x magnification using a Zeiss microscope. Analysis of scratch area reduction was done by Biomas software. Immunofluorescence microscopy was performed staining for E-Cadherin and CDK4.

3. Scratch assay of different primary cells

Primary cells of five different malign melanoma patients, as well as two different commercial breast cancer cell lines and one melanoma cell line (Mel624) were studied in a scratch assay under inhibitor and/or radiation treatment. As control we treated healthy skin fibroblasts in the same setting. Figure 2 depicts representative images of scratch closing within 48 hours of breast carcinoma cell line MCF-7.

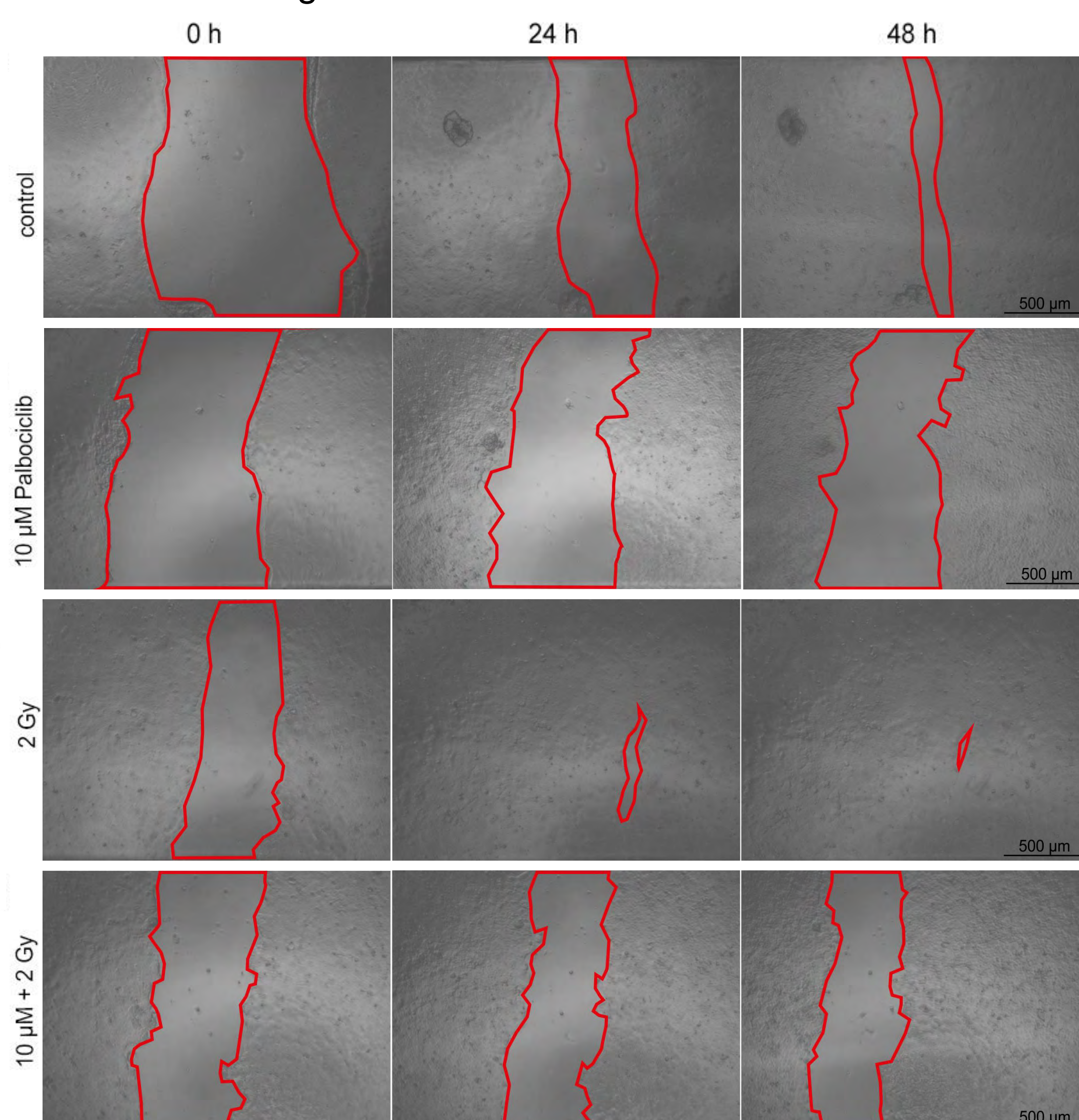


Fig.2: Representative images of scratch area during 48 h treatment with Palbociclib, irradiation or combination of both of breast cell line MCF-7.

4. Palbociclib inhibits cell migration

In all studied cells and cell lines Palbociclib slowed down the closing of the scratch area. Additionally irradiation seems to have no influence on migration behavior of the cells. Fastest closing was measurable for breast carcinoma cell line MDA-MB-231. Healthy skin fibroblasts SBLF9 migrated the slowest even without treatment.

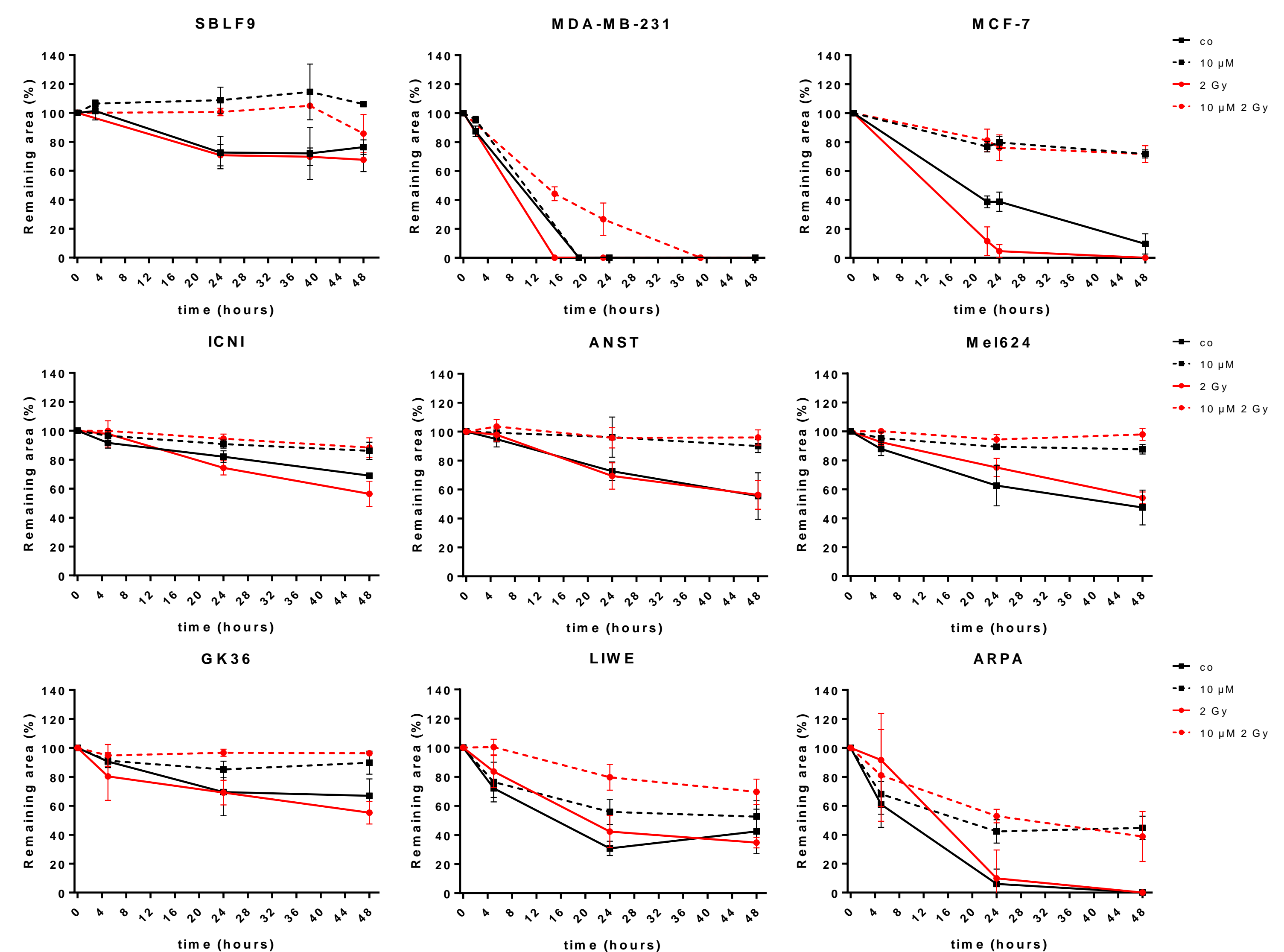


Fig.3: Scratch assay of different primary cells and cell lines under Palbociclib treatment, irradiation of 2 Gy or a combination therapy.

The cells of melanoma patients ICNI, ANST and GK36 as well as the melanoma cell line Mel624 migrated similar without scratch-closing within 48 h. LIWE migrated moderate and for ARPA scratch-closing was detectable during 48 h.

Differences in migration of the primary cells and further cell lines may due to the different expression of E-Cadherin, a cell adhesion protein. But comparing migration and expression of E-Cadherin did not show a correlation so far. ARPA cells migrated fast but showed equal E-Cadherin expression as slow migrating Mel624 cells.

CDK4 was detectable in all studied cells and there showed almost no differences in the treated and/or irradiated samples.

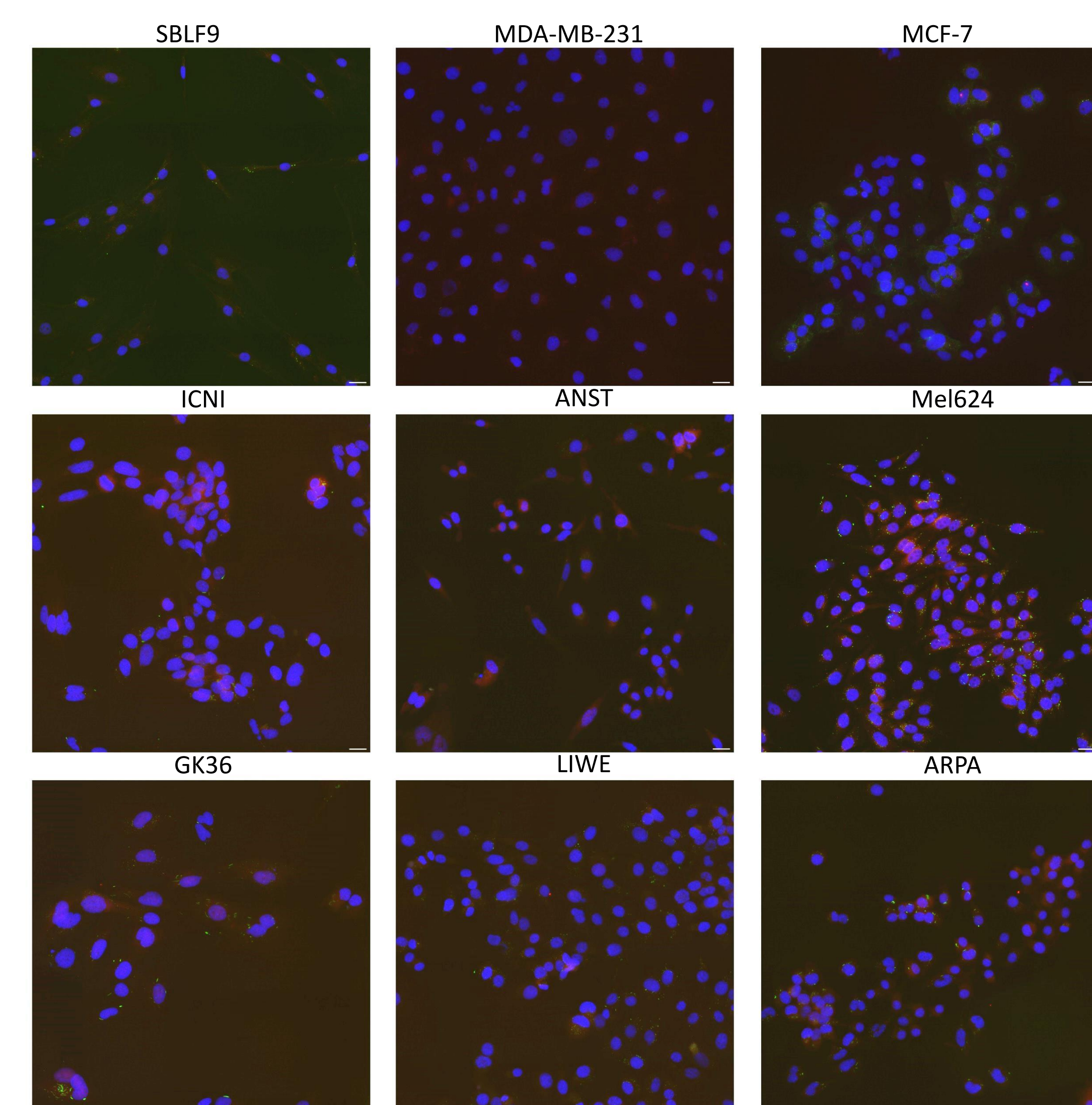


Fig.4: Immunofluorescence microscopy of different primary cells and cell lines for E-Cadherin (green) and CDK4 (red); DAPI (blue). Scale bars 25 µm.

5. Conclusion

Palbociclib is able to down-regulate migration of malign and non-malign cells. For breast cancer cell lines this may due to different E-Cadherin expression. However, staining of further primary cells of malign melanoma patients showed no correlation between migration velocity and E-Cadherin expression. Nevertheless, an earlier treatment could be more beneficial for breast cancer patients because of inhibitory influence of Palbociclib on migration.