Aalborg Universitet

Photonic modulation of EGFR

280nm low level light arrests cancer cell activation and migration

Botelho, Cláudia M.; Marques, Rogério; Viruthachalam, Thiagarajan; Gonçalves, Odete; Vorum, Henrik; Gomes, Andreia C.; Neves-Petersen, Maria Teresa

PROCEEDINGS OF SPIE

Photonic modulation of EGFR:
280nm low level light arrests cancer
cell activation and migration

Cláudia Manuela Botelho, Rogério Marques, Thiagarajan Viruthachalam, Odete   Gonçalves, Henrik Vorum, et al.

SPIE.

Photonic modulation of EGFR – 280nm low level light arrests cancer cell activation and migration

Cláudia Manuela Botelho[a], Rogério Marques[b], Viruthachalam Thiagarajan[c,d], Odete Gonçalves[e], Henrik Vorum[f], Andreia Castro Gomes*[b] and Maria Teresa Neves-Petersen[c,f]*

[a]Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal.
[b]Centre of Molecular and Environmental Biology (CBMA), Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[c]BioPhotonics Group, International Iberian Nanotechnology Laboratory (INL), P-4715-310 Braga, Portugal.
[d]School of Chemistry, Bharathidasan University, Tiruchirappalli-620 024, India
[e]Department of Health Science and Technology, Aalborg University, Fredrik Bajers Vej 7, 9220 Aalborg, Denmark
[f]Department of Clinical Medicine, Aalborg University Hospital, Hobrovej 18-22, 9000 Aalborg, Denmark.

ABSTRACT

Overexpression of the Epidermal Growth Factor Receptor (EGFR) by cancer cells is associated with a poor prognosis for the patient. For several decades, therapies targeting EGFR have been designed, including the use of monoclonal antibodies and small molecule tyrosine kinase inhibitors. The use of these molecules had good clinical results, although its efficiency (and specificity) is still far from being optimal.

In this paper, we present a new approach for a possible new cancer therapy targeting EGFR and using low intensity 280nm light. The influence of 280nm UVB illumination on cancer cells stimulated with 2nM of EGF was followed by time-lapse confocal microscopy. The 280nm illumination of the cancer cells blocks EGFR activation, inhibiting EGFR internalization and cell migration thus inhibiting the transition to the metastatic phenotype. Exposure time is a very important factor. The higher the illumination time the more significant differences were observed: 280nm light delayed or completely halted EGFR activation in the cell membrane, mainly at the cell junction level, and delayed or halted EGFR endocytic internalization, filopodia formation and cell migration.

Keywords: Epidermal Growth Factor Receptor, Activation, Arrest, EGFR, EGF, Cancer cells, Photonic Therapy, Cell Migration, Filopodia.

1. INTRODUCTION

Solid tumor growth is associated to several genetic events that influence cell differentiation, proliferation, survival and mobility[1]. The activation of the pro-oncogene encoding for the epidermal growth factor receptor (EGFR) confers advantages to the cancer cells promoting survival and proliferation[1]. A high level of EGFR expression by cancer cells is associated with a poor prognosis for the patient. EGFR is overexpressed in most solid tumors, such as, breast, head and neck, non-small cell lung cancer and prostate cancer[2,3]. During the past decades several therapies targeting EGFR have been designed using monoclonal antibodies[4-9] and small molecule tyrosine kinase inhibitors[5,8,10-13]. These approaches have achieved some good results, although its efficiency is still far from being optimal[14,15].

The development of an efficient anti-cancer therapy, which can be used alone or as a complementary technique, is of outmost importance. Photonics, more precisely biophotonics, is a promising field in this regard. In 1903, Niels Ryberg was awarded with the Nobel Prize in Medicine and Physiology due to outstanding and extensive use of UV light in the treatment of skin diseases. Currently, medical treatments using UV light are quite common. UV light has been applied for the treatment of skin diseases like psoriasis[16,17]. Additionally, UV light is used to treat cutaneous T-cell lymphoma[18,19], a therapy approved by the Food and Drug Administration (FDA). The use of photons for cancer treatment is not a novel concept but new approaches are being explored. It is known that UVB light affects proteins. UVB excitation of tryptophan (Trp) and tyrosine (Tyr) side chains leads to the reduction of disulphide bridges bridges in a protein[20,21]. UV excitation of Trp or Tyr residues results in photoionization and generation of solvated electrons[21,22]. The solvated electrons undergo fast geminate recombination with their parent molecule, or they can be captured by electrophilic species like molecular oxygen, H_3O^+ (at low pH), and cysteines (schemes 1, 2 and 3, respectively):

$$e_{aq}^- + O_2 \rightarrow O_2^{\bullet-} \tag{1}$$
$$e_{aq}^- + H_3O^+ \rightarrow H^\bullet + H_2O \tag{2}$$
$$e_{aq}^- + RSSR \rightarrow RSSR^{\bullet-} \tag{3}$$
$$RSSR^{\bullet-} \Leftrightarrow RS^\bullet + RS^- \tag{4}$$
$$RSSR^{\bullet-} + H^+ \Leftrightarrow RS^\bullet + RSH \tag{5}$$

The electron captured by the cysteine (scheme 3) leads to the breakage of the SS bridge (schemes 4 and 5). EGFR is the perfect target for this approach, as it is rich in disulphide bridges in close spatial proximity of aromatic residues: it has 25 disulphide bridges, 6 Trp residues, 16 Tyr residues and 18 Phe residues per chain. This feature makes EGFR extremely susceptible to 3D conformational changes induced by UV light. The induced conformational changes will prevent the correct binding to its ligands, inhibiting its function. Olsen et al.[23] demonstrated that laser pulsed UVB (280nm) excitation of two skin-derived tumor cell lines overexpressing EGFR, A431 (human epidermoid carcinoma cells) and Cal39 (derived from human vulva squamous cell carcinoma cells), with an irradiance level of 0.35 W.m^{-2} leads to the inhibition of the EGF receptor and of key downstream molecules such as AKT1 and ERK1/2 involved in the RTK-catalyzed signaling cascade, leading to cell apoptosis. Correia et al.[24] demonstrated that illumination of EGFR with UVB light (280 nm) at an irradiance level of 0.08 W.m^{-2} induced structural changes in the receptor affecting the EGF binding site. More recently, Botelho et al.[25] demonstrated that the illumination of pulmonary cancer cells with UVB light using an irradiance of 0.27 W.m^{-2} maintains the integrity of the confluent cancer cell monolayer upon EGF activation for a longer period of time in comparison with non-illuminated cancer cells.

The present study aims at evaluating the influence of 280nm illumination time on cancer cell migration, using a fluency of 1.18 W.m^{-2}. This value is lower than the highest total irradiance of sunlight in the UVB region is ~0.78W.m^{-2} in the range 280-315nm[26], which is, and 1.75W.m^{-2} at 39ºN in summer and 0.4 W/m^2 in December at wavelengths below 313nm[27].

2. MATERIAL AND METHODS

2.1. Cell line

The cell line used in this study was the lung carcinoma cell line with GFP-tagged EGFR (A549-GFP-tagged SH2 biosensor cell line from Sigma-Aldrich, 86012804), which expresses EGFR tagged with GFP. The EGFR gene was endogenously tagged with a Green Fluorescent Protein gene (GFP) using CompoZr® Zinc Finger Nuclease technology. Cells were seeded into μ-Slide 4 wells (3×10^4 cells/cm^2) in Dulbecco's Modified Eagle Medium (DMEM) with 2 mM glutamine, 1 μg/mL puromycin (complete medium) and 10% of fetal bovine serum (FBS). The cancer cells were kept at 37ºC in a 5% CO_2 atmosphere in complete medium, 10% of FBS. At the beginning of each experiment the culture medium was replaced by DMEM containing 2% of FBS and EGF (0 nM and 2 nM).

2.2. Illumination setup (280 nm)

280nm illumination was provided by the Xe arc lamp (LX 300UV) of an ISS Chronos BH steady state fluorometer with a computer controlled monochromator. The light was focused onto an optical fiber with a core diameter of 150μm. Light was shaped to a 1cm diameter circular beam and passed centrally through the excitation volume. The cells were illuminated for 15min and 45min with 280nm light at 1.18 W.m^{-2} (0.093mW, 18A, slit width 16nm). The solution was kept at room temperature (~22ºC) during illumination.

2.3. Confocal Laser Scanning Microscopy (CLSM)

The morphology and the mobility of non-illuminated *versus* 280nm illuminated cells were monitored using CLSM. All experiments were carried out at 37ºC using a heat chamber coupled onto the Zeiss-LSM780. The cell medium containing 2% FBS was supplemented with 2nM EGF when appropriate. The addition of EGF was considered the time zero for the time-lapse experiments

3. RESULTS AND DISCUSSION

Cancers originated in epithelial cells (carcinomas) represent about 85% of all cancers. According to the literature, at least 33-50% of human carcinomas have a significant overexpression of EGFR[28–30]. As previously mentioned, the success of the existing anti-cancer therapies targeting EGFR is still far from optimal. Therefore, in this paper we describe a new method to target EGFR, disrupting its structure and function, which can be used as a complementary technique to the existing ones. The beneficial effects of UVB light are widely described in the literature, from the stimulation of vitamin D production to the treatment of skin diseases like, psoriasis, vitiligo, atopic dermatitis and localized scleroderma[31-33].

The ErbB family of receptors, which includes EGFR, influences cell survival, adhesion, migration, proliferation and differentiation[28]. Thus, it was expected that stimulation of cells overexpressing EGFR with 2nM of EGF would result in high cell migration and EGFR dimerization and consequent receptor activation. Cell morphology and behavior were followed by time-lapse using confocal microscopy.

The first step of this study was to evaluate the morphology and behavior of the cells for a period of 60 minutes without any EGF stimulation or UVB light illumination. As it can be seen in Figure 1, when non-activated and non-illuminated cells are monitored for 60 minutes there are no significant changes on cell morphology and it is possible to observe the early stage of cell migration (Figure 1 A5). When these cells were stimulated with 2nM of EGF, a significant number of events take place as it can be seen in Figure 2. The activation of EGFR receptors is observable 6 min after stimulation by EGF (Figure 2 A2), especially in cell junctions, followed by the internalization through endocytosis, which is visible by the appearance of bright fluorescent dots inside the cell 14 min after EGF stimulation. This event is even more evident 28 min after EGF stimulation (Figure 2 A4). Finally, after 60 min, the disintegration of the cell monolayer is evident, as well as filopodia formation and internalization of the EGF-EGFR complexes. These results are in agreement with previous studies regarding the intracellular trafficking of EGFR[34], showing that EGFR signaling continues after receptor internalization. The effects observed are consistent with the acquisition of a metastatic phenotype which is correlated with the epithelial-mesenchymal transition (EMT)[35-38]. EMT is associated with E-cadherin downregulation affecting the integrity of the adherent junctions[39]. EGFR signaling may lead to the loss of cell-cell adhesion through E-cadherin downregulation. This may be due to the destabilization of E-cadherin/catenin adhesive complexes, E-cadherin endocytosis or downregulation of E-cadherin expression. Additionally, EMT is accompanied by downregulation of P-cadherin and cadherin 6, as well as the appearance of N-cadherin, cadherin 11 and R-cadherin. Therefore, E-cadherin can be considered an invasiveness suppressor gene[39].

Figure 1 – Confocal laser scanning microscopy images of lung carcinoma cells. A1 – Cell monolayer morphology before EGF addition; A2-5 – Morphology of cell monolayer after 6, 14, 28 and 60min, respectively (EGF has not been added to the medium).
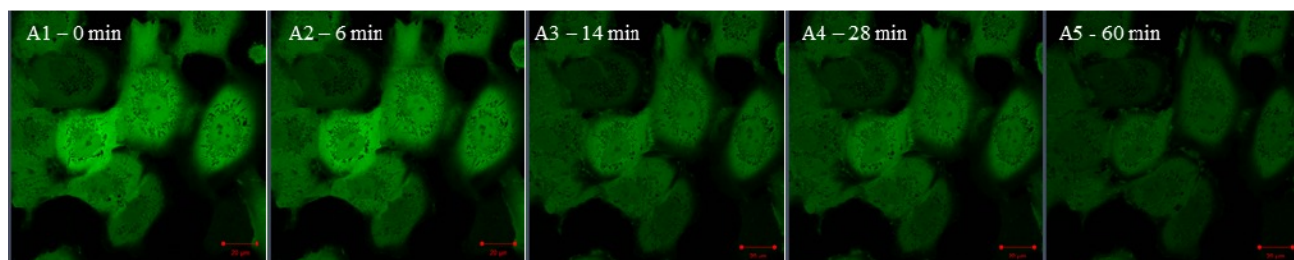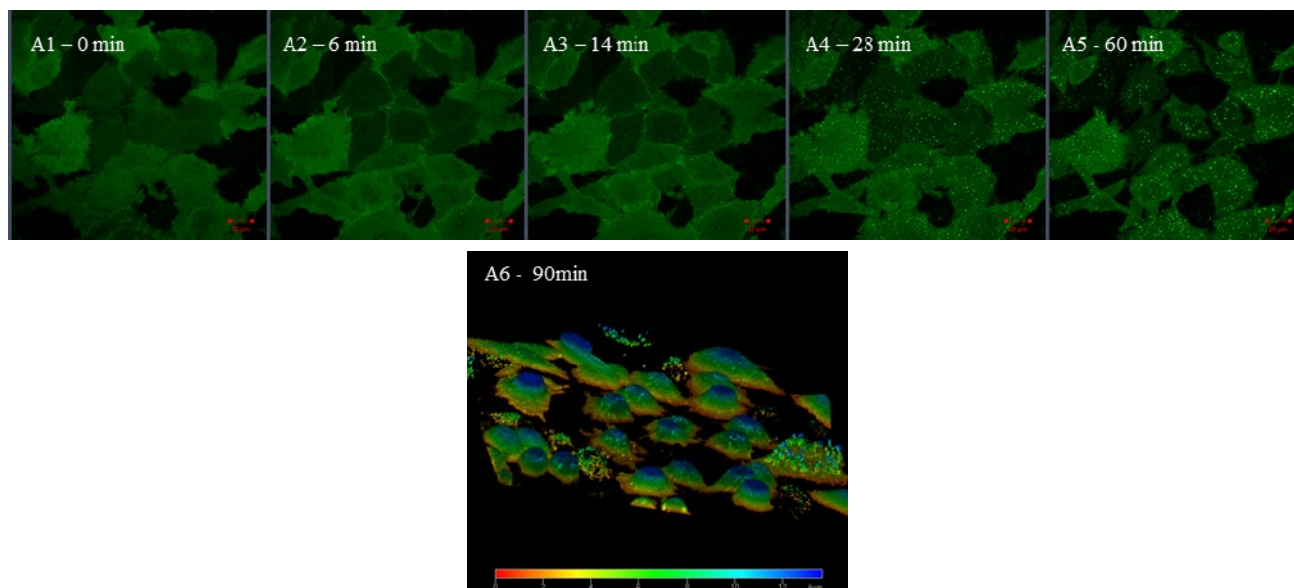

Figure 2 - Confocal laser scanning microscopy images of lung carcinoma cells. A1 – Cell monolayer morphology before EGF addition; A2-5 – Morphology of cell monolayer after 6, 14, 28 and 60min exposure to 2nM of EGF, respectively; A6 – 3D image of the cell monolayer after 90min of exposure to 2nM of EGF. The scale bar represents the depth in the Z axis.

The second step of this study was to evaluate the effect of 280nm light on non-stimulated cells. For that, the cells were illuminated with 280nm at an irradiance level of 1.18 W.m^{-2} using two different exposures time, 15 and 45min. As can be seen in Figures 3 and 4, no significant changes on the morphology of the cells were observed from time 0 to time 60min after EGF addition, in either case. The illumination of the non-stimulated cells seems to delay cell migration when the cells were illuminated with 280nm light for 15 or 45 min, as can be observed when comparing the 60min panels (Figures 3 and 4, panelsA5) with the respective data displayed in Figure 1 A5.
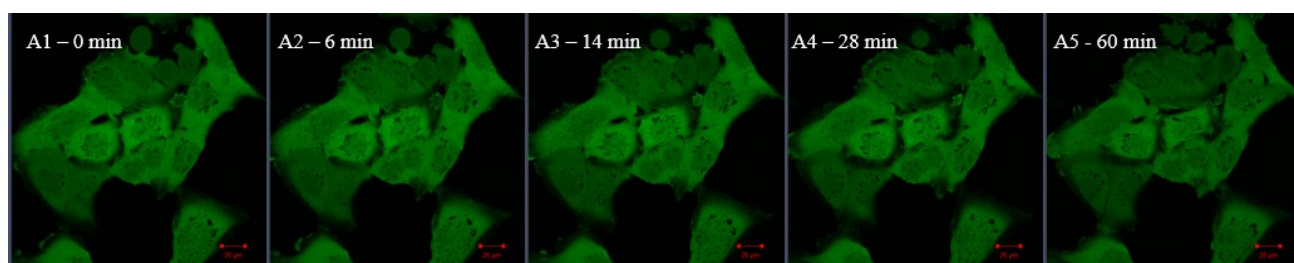

Figure 3 - Confocal laser scanning microscopy images of lung carcinoma cells. A1 – Cell monolayer morphology before EGF addition; A2-5 – Morphology of cell monolayer after 6, 14, 28 and 60min. 280nm illumination has been carried out with an irradiance of 1.18 W.m-2 for 15min. No EGF has been added to the cells in this experiment.
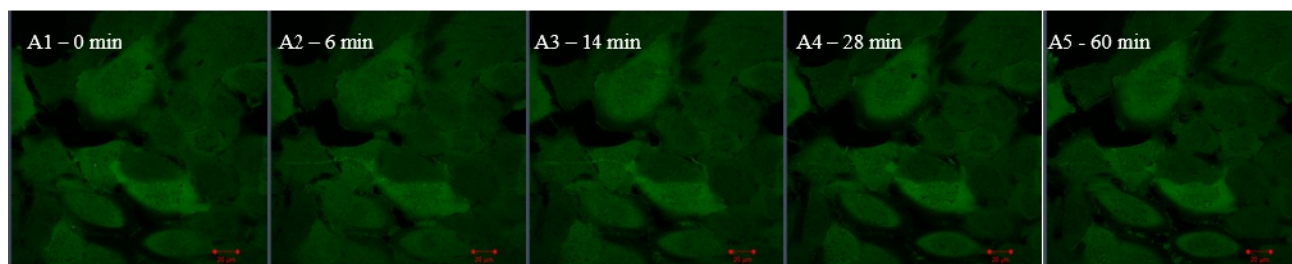
Figure 4 - Confocal laser scanning microscopy images of lung carcinoma cells. A1 – Cell monolayer morphology before EGF addition; A2-5 – Morphology of cell monolayer after 6, 14, 28 and 60min. 280nm illumination has been carried out with an irradiance of 1.18 W.m-2 for 45min. No EGF has been added to the cells in this experiment.

The final goal of this new biophotonic approach was to delay migration of EGF-stimulated cells. Time-lapse confocal microscopy was used to evaluate the influence of 280nm light on cancer cells stimulated with 2nM of EGF. As observed in Figure 5, when the stimulated cells were exposed to 280nm light for 15 min, EGFR activation is only observed 14-28min after EGF addition. The internalization of these complexes is more evident at 60 min. The formation of filopodia is scarce and no evident cell migration is observed. The internalization of EGFR/EGF complexes can be observed and is displayed as a 3D image (Figure 5 A6) obtained 90min after EGF stimulation but at a lower extent than in non-illuminated cells.
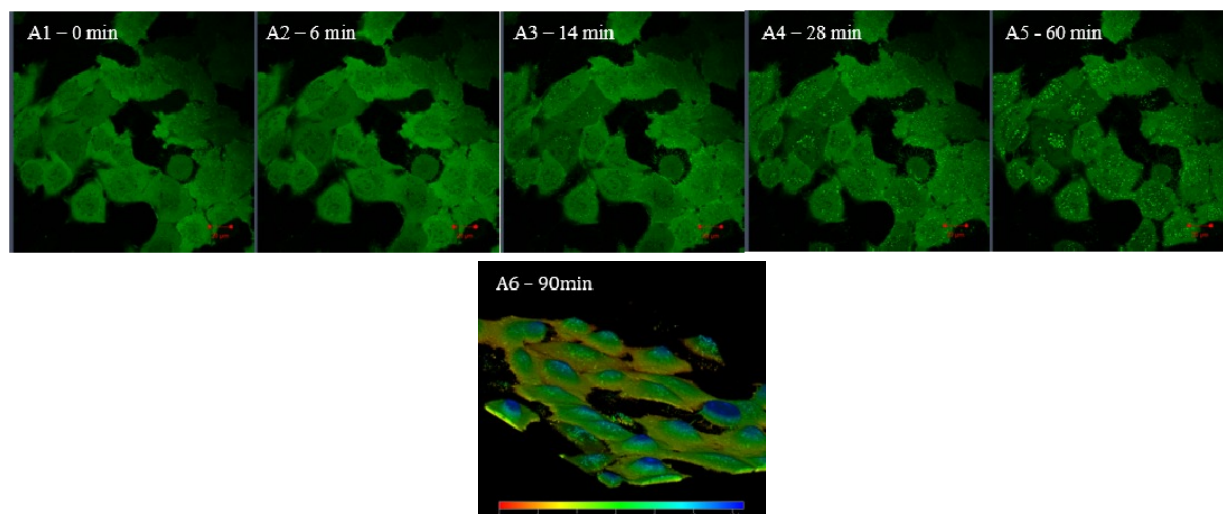

Figure 5 - Confocal laser scanning microscopy images of lung carcinoma cells. A1 – Cell monolayer morphology before EGF addition; A2-5 – Morphology of cell monolayer after 6, 14, 28 and 60min exposure to 2nM of EGF, respectively. Prior to EGF addition, the cells have been illuminated with an irradiance of 1.18 W.m-2 for 15min; A6 – 3D image of the cell monolayer after 90min of exposure to 2nM of EGF. Prior to EGF addition, the cells have been illuminated with 280nm at an irradiance of 1.18 W.m-2 for 15min.

The delayed activation of EGFR by EGF and consequent receptor internalization is even more evident in cells that were illuminated for a longer period of time (45 min) prior to EGF stimulation. As it can be observed in Figure 6 A4, the extent of EGFR activation observed 28min after EGF stimulation in cells previously illuminated for 45min is less than the respective level of activation observed in stimulated but non-illuminated cells (Figure 2 A4) and less than that of cells illuminated for only 15 min (Figure 3 A4). This is even more clear when looking at the confocal images obtained 60 min after EGF stimulation. The integrity of the cell monolayer is practically intact in cells previously illuminated for 45min with 280nm light at 1.18 W.m^{-2}.
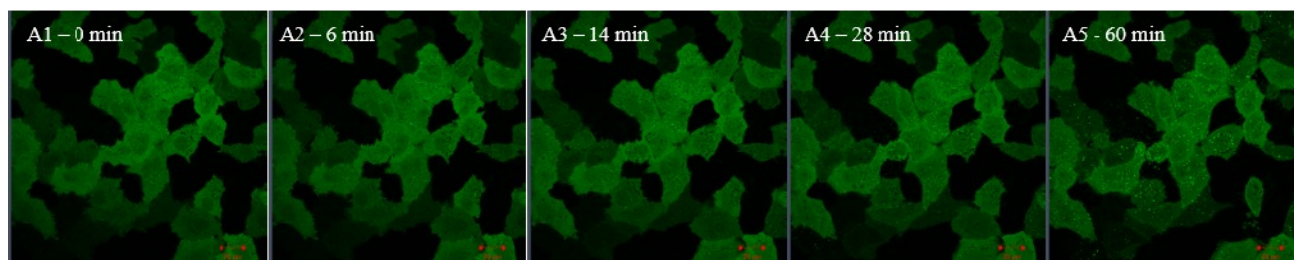
Figure 6 - Confocal laser scanning microscopy images of cancer cells. A1 – Cell monolayer morphology before EGF addition; A2-5 – Morphology of cell monolayer after 6, 14, 28 and 60min exposure to 2nM of EGF, respectively. Prior to EGF addition, the cells have been illuminated with 280nm with an irradiance of 1.18 W.m-2 for 45min.

The exposure time is indeed a very important factor in halting EGFR activation, and internalization, and cellular migration, as can be observed in Figure 7. Figure 7 shows the clear influence of 280nm light and exposure time on EGFR activation, internalization, filopodia formation and cell migration (Figure 7 A4, B4 and C4). It can be observed that the illumination of cells prior to EGF stimulation delays these events, being the effect more evident in cells illuminated for a longer period of time.
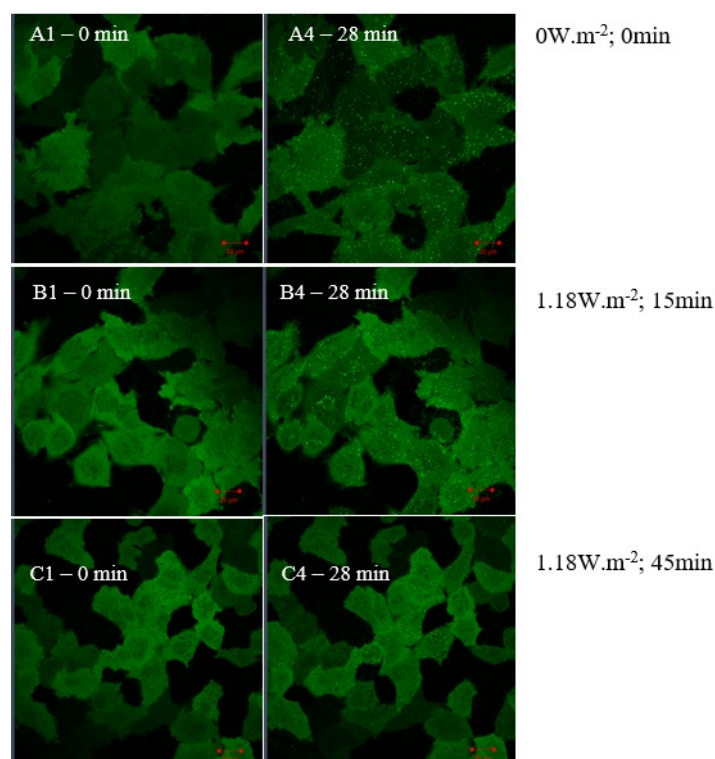

Figure 7 - Confocal laser scanning microscopy images of lung carcinoma cells. A1, B1 and C1 – Cell monolayer morphology before EGF addition; A4, B4 and C4 – Morphology of cell monolayer after 28min exposure to 2nM of EGF - Prior to EGF addition, the cells have been illuminated with 280nm with an irradiance of 0W.m-2 and 1.18 W.m-2 for 15min and 45min, respectively.

4. CONCLUSION

The results presented in this paper demonstrated that 280nm illumination of EGFR overexpressing cells prior to their stimulation with EGF affects the EGFR/EGF binding ability and, consequently, receptor activation. The 280nm illumination time is an important factor, as longer illumination more strongly impairs the activation of EGFR by EGF ligand. Filopodia formation and cell migration is significantly delayed when the pulmonary cancer cells are illuminated

with 280nm light. Blocking the loss of cellular adhesion and motility is crucial to prevent tumour initiation and progression. The new photonic approach has several advantages: it is non-invasive, low intensity of light is used, the illumination time is short, no photosensitizer drugs or any other drug are needed. Light can reach deeper tissues through the use of optical fibers and/or multiphoton excitation. The biophotonic approach presented in this paper opens the doors to the development of a promising anti-cancer therapy.

5. ACKNOWLEDGMENTS

6. REFERENCES

[1]   Nicholson, R.I, Gee, J.M. and Harper, M.E.," EGFR and cancer prognosis," Eur J Cancer 37 Suppl 4:S9-S15 (2001).

[2]   Salomon, D.S., Brandta, R., Fortunato, C. and Normannoc, N., " Epidermal growth factor-related peptides and their receptors in human malignancies," Crit Rev Oncol Hematol. 19:183-232 (1995).

[3]   Ciardiello, .F, and Tortora, G., "Epidermal growth factor receptor as a target in cancer therapy: understanding the role of receptor expression and other molecular determinants that could influence the response to to anti-egfr drugs," Eur J Cancer 39:1348-1354 (2003).

[4]   Mendelsohn and J., Baselga, J., "The EGF receptor family as targets for cancer therapy," Oncogene 19(56):6550-65 (2000).

[5]   Ahdi Khosroshahi, S., Farajnia, S., Ghiamirad, M., Tanomand, A., Veisi, K., Rahbarnia, L. and Akbari, B, " Development and evaluation of a single domain antibody against human epidermal growth factor receptor (EGFR), " Protein Expr Purif.120:59-64 (2016).

[6]   Schlessinger, J., Schreiber, A.B., Levi, A., Lax, I., Libermann, T. and Yarden, Y., "Regulation of cell proliferation by epidermal growth factor," CRC Crit Rev Biochem 14(2):93-111 (1983).

[7]   Yarden, Y. and Shilo, B-Z., "SnapShot: EGFR signaling pathway. Cell 131(5):1018 (2007)..

[8]   Herbst, R.S., "Review of epidermal growth factor receptor biology," Int J Radiat Oncol Biol Phys. 59(2 Suppl):21-6 (2004).

[9]   Verstraete, M., "Third-generation thrombolytic drugs," Am J Med. 109(1):52-8 (2000).

[10]   Mlika, M., Laabidi, S., Afrit, M., Boussen, H. and El Mezni, F., "Genomic classification of lung cancer: toward a personalized treatment," Tunis Med 93(6):339-44 (2015).

[11]   Hegi, M.E., Rajakannu, P. and Weller, M., "Epidermal growth factor receptor: a re-emerging target in glioblastoma." Curr Opin Neurol 25(6):774-9 (2012).

[12]   Wheeler, D.L., Dunn, E.F. and Harari, P.M., "Understanding resistance to EGFR inhibitors-impact on future treatment strategies," Nat Rev Clin Oncol 7(9):493-507 (2010).

[13]   Zhang, H., Berezov, A., Wang, Q., Zhang, G., Drebin, J., Murali, R. and Greene, M., "ErbB receptors: from oncogenes to targeted cancer therapies," J Clin Invest 117(8):2051-8 (2007).

[14]   Hojjat-Farsangi, M., "Small-molecule inhibitors of the receptor tyrosine kinases: promising tools for targeted cancer therapies," Int J Mol Sci 15(8):13768-13801 (2004).

[15]   Ono, M. and Kuwano, M. "Molecular mechanisms of epidermal growth factor receptor (EGFR) activation and response to gefitinib and other EGFR-targeting drugs," Clin Cancer Res 12(24):7242-7251 (2006).

[16]   Reich, A. and Medrek, K. "Effects of narrow band UVB (311 nm) irradiation on epidermal cells," Int J Mol Sci 14(4):8456-8466 (2013).

[17]   Berneburg, M., Röcken, M. and Benedix, F., "Phototherapy with narrowband vs broadband UVB," Acta Derm Venereol 85(2):98-108 (2005).

[18]   Poligone, B. and Heald, P., "Innovative therapy of cutaneous T-Cell lymphoma: Beyond psoralen and ultraviolet light and nitrogen mustard," Dermatol Clin 28(3):501-510 (2010).

[19]   Kacinski, B.M and Flick, M., "Apoptosis and cutaneous T cell lymphoma," Ann N Y Acad Sci 941:194-9 (2001).

[20]   Neves-Petersen, M.T., Gryczynski, Z., Lakowicz, J., Fojan, P., Pedersen, S., Petersen, E. and Petersen, S.B., "High probability of disrupting a disulphide bridge mediated by an endogenous excited tryptophan residue," Protein Sci 11(3):588-600 (2002).

[21]   Neves-Petersen, M.T., Klitgaard, S., Pascher, T., Skovsen, E., Polivka, T., Yartsev, A., Sundstöm, V. and Petersen, S.B., "Flash photolysis of cutinase: Identification and decay kinetics of transient intermediates formed upon UV excitation of aromatic residues," Biophys J 97(1):211-226 (2009).

[22]   Kerwin, B.A. and Remmele, R.L., "Protect from light: Photodegradation and protein biologics," J Pharm Sci 96(6):1468-1479 (2007).

[23]   Olsen, B.B., Neves-Petersen, M.T., Klitgaard, S., Issinger, O.G. and Petersen, S.B. "UV light blocks EGFR signalling in human cancer cell lines," Int J Oncol 30(1):181-5 (2007).

[24]   Correia, M., Thiagarajan, V., Coutinho, I., Gajula, G.P., Petersen, S.B. and Neves-Petersen, M.T., "Modulating the Structure of EGFR with UV Light: New Possibilities in Cancer Therapy," PLoS One 9(11):e111617 (2014).

[25]   Botelho, C., Thiagarajan, V., Marques, R., Petersen, S.B., Gomes, A., Vorum, H. and Neves-Petersen, M.T., "Towards a new photonic cancer therapy: Stopping cancer cell activation, migration and metastases using light" Int J Mol Med (405):36(Suppl. 1):S81 (2015).

[26]   ASTM Subcommittee G03.09 (2003) ASTM G173–03 Standard Tables for Reference Solar Spectral Irradiance Direct Normal and Hemispherical on 37° Tilted Surface. Annual Book of ASTM Standards. West Conshohocken, PA: ASTM International, Vol. 14.04. - Open Access. Available at: http://www.oalib.com/references/11745669. Accessed February 1, 2016.

[27]   Pirie, A. and Dilley, K.J. " Photo-oxidation of N'-Formylkynurenine and Tryptophan peptides by sunlight or simulated sunlight," Photochem Photobiol 19(2):115-118 (1974).

[28]   Yarden, Y. and Sliwkowski, M.X., "Untangling the ErbB signalling network" Nat Rev Mol Cell Biol 2(2):127-37 (2001).

[29]   Polsky, D. and Cordon-Cardo, C., "Oncogenes in melanoma," Oncogene 22(20):3087-91 (2003).

[30]   Woodburn, J.R. "The epidermal growth factor receptor and its inhibition in cancer therapy," Pharmacol Ther 82(2-3):241-50 (1999).

[31]   Juzeniene, A. and Moan, J., "Beneficial effects of UV radiation other than via vitamin D production," Dermatoendocrinol 4(2):109-17 (2012).

[32]   Lapolla, W., Yentzer, B.A., Bagel, J., Halvorson, C.R., Feldman, S.R., "A review of phototherapy protocols for psoriasis treatment" J Am Acad Dermatol 64(5):936-949 (2011).

[33]   Gambichler, T., Breuckmann, F., Boms, S., Altmeyer, P. and Kreuter, A., "Narrowband UVB phototherapy in skin conditions beyond psoriasis," J Am Acad Dermatol 52(4):660-670 (2005).

[34]   Sorkin, A. and Goh, L.K., "Endocytosis and intracellular trafficking of ErbBs" Exp Cell Res 315(4):683-96 (2009).

[35]   Thiery, J.P., "Epithelial–mesenchymal transitions in development and pathologies," Curr Opin Cell Biol 15(6):740-746 (2013).

[36]   Kang, Y., Massagué, J.. "Epithelial-mesenchymal transitions: twist in development and metastasis," Cell 118(3):277-9 (2004).

[37]   Yilmaz, M., Christofori, G., "EMT, the cytoskeleton, and cancer cell invasion," Cancer Metastasis Rev 28(1-2):15-33 (2009).

[38]   Iwano, M., Plieth, D., Danoff, T.M., Xue, C., Okada, H. and Neilson, E.G., "Evidence that fibroblasts derive from epithelium during tissue fibrosis" J Clin Invest 110(3):341-350 (2002).

[39]   Pećina-Slaus, N., "Tumor suppressor gene E-cadherin and its role in normal and malignant cells," Cancer Cell Int 3(1):17 (2003).