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Results

Sensitivity of the assay in NGS and AS-PCR

Mutant allele frequencies (MAF) were analyzed by NGS in wild-type DNA and in cfDNA of 12 healthy individuals. The median MAF varied from 0 to 0.77% in wild type DNA and from 0 to 0.44% in healthy individuals as calculated on the basis of reads amounts. There were no statistically significant differences between the baseline MAF in wild type genome DNA and cfDNA from healthy individuals. According to this data the sensitivity of the assay was 0.77%. AS-PCR data were validated with the control DNA and in the majority of cases the provided sensitivity of the assay was in range of 1%.

Testing of clinical samples of plasma cfDNA

87 plasma samples from 14 patients with NSCLC were analyzed. Four patients had mutation EGFR T790M associated with resistance to TKI before the start of the therapy. Monitoring of cfDNA in plasma of 14 patients with NSCLC revealed various dynamic profiles of EGFR mutations associated with sensitivity and resistance to TKI. We analyzed the period of 18 months for the majority of patients with a 2-month interval. At least 5 patients were found where NGS and AS-PCR data were in agreement. In all those cases the dynamics of mutations were different. However, at least in two cases an obvious increase of DNA fragments with T790M mutation was observed well before clinical symptoms appearance.

Conclusion

This study highlights the utility of cfDNA for analysis EGFR mutations in blood of NSCLC patients being treated by TKI. The level of sensitivity appeared to be comparable between NGS and AS-PCR.

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REFERENCES

1. *Suda K., Mitsudomi T.* Role of EGFR mutations in lung cancers: prognosis and tumor chemosensitivity. Arch Toxicol. 2015; 89(8): 1227-40. doi: 10.1007/s00204-015-1524-7.

2. *Sakai K., Horiike A., Irwin D.L., Kudo K., Fujita Y., Tanimoto A., Sakatani T., Saito R., Kaburaki K., Yanagitani N., Ohyanagi F., Nishio M., Nishio K.* Detection of epidermal growth factor receptor T790M mutation in plasma DNA from patients refractory to epidermal growth factor receptor tyrosine kinase inhibitor. Cancer Sci. 2013 Sep; 104(9): 1198-204. doi: 10.1111/cas.12211.

3. *Li W., Qiu T., Guo L., Ling Y., Gao Y., Ying J., He J.* Primary and acquired EGFR T790M-mutant NSCLC patients identified by routine mutation testing show different characteristics but may both respond to osimertinib treatment. Cancer Lett. 2018 Jun 1; 423: 9-15. doi: 10.1016/j.canlet.2018.03.005.

4. *Francis G., Stein S.* Circulating Cell-Free Tumour DNA in the Management of Cancer. Int J Mol Sci. 2015 Jun 19; 16(6): 14122-42. doi: 10.3390/ijms160614122.

5. *Li H., Durbin R.* Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009 Jul 15; 25(14): 1754-60. doi: 10.1093/bioinformatics/btp324.

EFFECT OF BIODEGRADABLE SCAFFOLDS FOR TARGETED IMMUNOTHERAPY ON DIFFERENTIATION OF MACROPHAGES

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The influence of polycaprolactone's scaffolds on the mechanisms of differentiation of primary monocytic human macrophages *in vitro* was studied.

Keywords: polycaprolactone, bone implants, bioresorbable scaffold.

Relevance

Traditional methods of treatment of malignant tumors include surgery, radiation and chemotherapy, which have reached the limit of their technical perfection to-date, and their further development will not lead to a significant improvement in the situation. The most actual of alternative therapies is personalized targeted immunotherapy, the realization of which requires targeted drug delivery. Polymeric delivery systems allow transporting drugs during the optimal period of time to a local site of an organ or tissue. The biodegradable polycaprolactone is used in medicine as a suture material and as a thermal bioresorbable implant depot (filler), so it can be used as capsule shells and for drugs. However, polymers have such a disadvantage as hydrophobicity, which prevents

adhesion of cells and their directional migration. Thus, a modification of the surface properties of polycaprolactone is necessary.

The aim of the study is to evaluate the influence of scaffolds based on polycaprolactone with a surface modified by plasma on the differentiation's mechanisms of primary monocyte macrophages *in vitro* [1]. Depending on the surface treatment time (0, 30, 60, 120, 240 seconds), the samples designated as № 1, 2, 3, 4, 5.

The studies were performed on human monocytes isolated from the buffy coat of a healthy human donor. The cells were isolated by magnetic separation on a double ficoll gradient of different densities using magnetic assays conjugated to antibodies to the CD14+ [2].The samples were placed on the bottom of a 24-well plate, isolated monocytes were added at a concentration of 1×10^6 in 1 ml. For each coating 3 experimental groups were formed: control (non-stimulated), with the addition of IFNγ (M1 activation) and with IL-4 (M2 activation). To assess the functional phenotype and the direction of differentiation of macrophages after 6 days of incubation, the cells were stained. In study antibodies: CD68 (mouse monoclonal), Stabilin-1 (RS1, rabbit polyclonal), CD206 (rabbit polyclonal) were used. As secondary antibodies Alexa488-conjugated anti-mouse and Cy3-conjugated anti-rabbit antibodies were used. The combinations of antibodies were verified: CD68/RS1 and CD68/CD206. The analysis of double positive cells was performed using a Carl Zeiss laser scanning microscope at a 100-fold magnification.

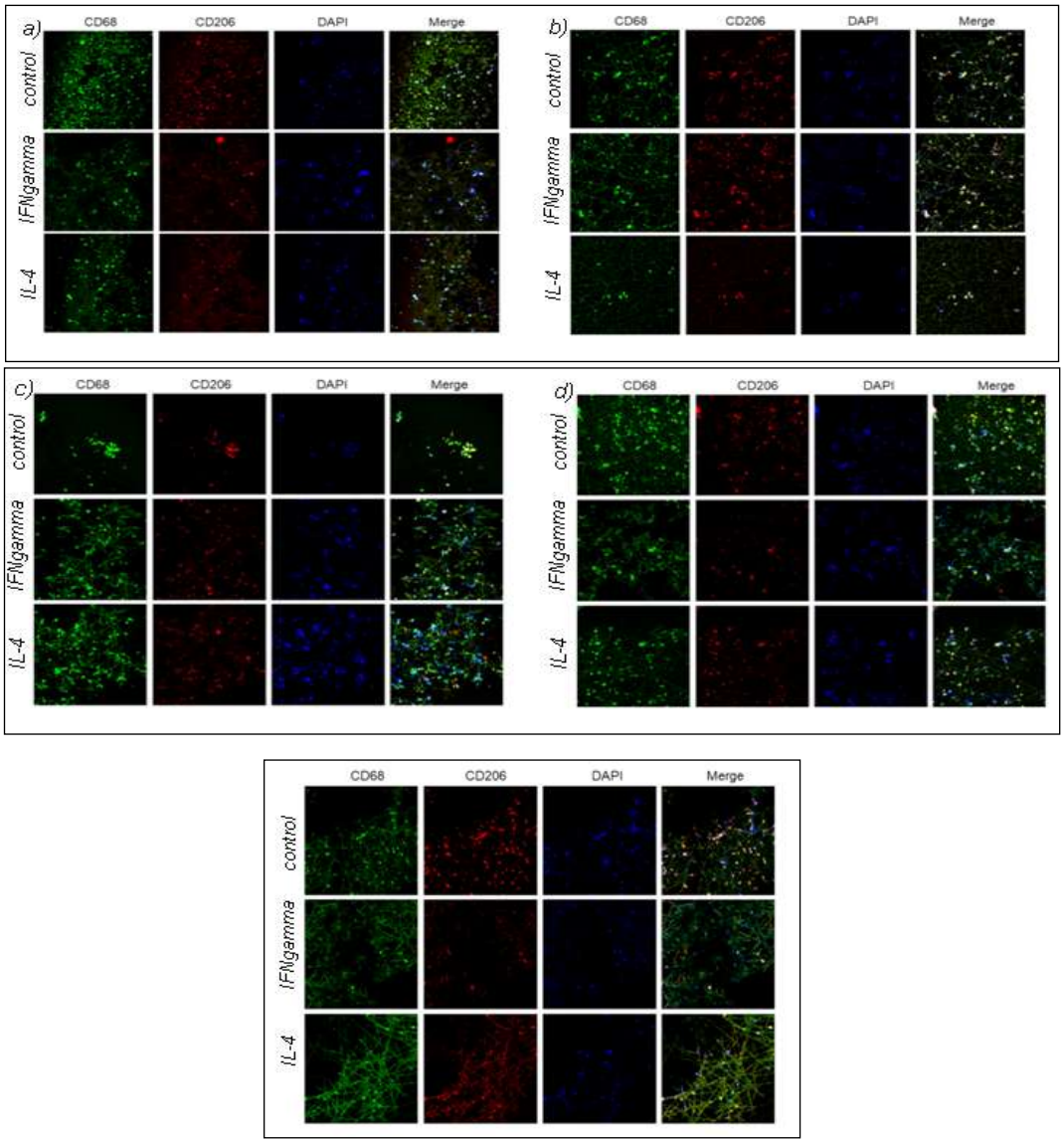

Figure 1. Analysis of CD68/CD206 phenotype macrophage on the sample surface: a – 1, b – 2, c – 3, d – 4, e – 5.

The analysis showed that in all groups after 6 days of cultivation all cells were M2 macrophages phenotype: CD68+/stabilin-1+ excluding group with sample 2. In this case, non stimulated cells

(M0 macrophages) and with the addition of IFNγ (M1 macrophages) had the phenotype CD68+/ stabilin-1. Study of CD206-positive cells showed that after the 6 days of incubation all cells in groups had a double positive color of CD68 +/CD206+ – subpopulation of M2 macrophages. Thus, macrophages cultured on scaffolds express on their surface scavenger receptors with the function of regulation of the intensity of the immune response and acquire a functional phenotype of anti-inflammatory macrophages M2 with immunosuppressive properties. This allows us to conclude that scaffolds have tolerogenic and anti-inflammatory properties. Samples 4 and 5 have the strongest stimulating effect on the expression of stabilin-1 in all three subpopulations of macrophages.

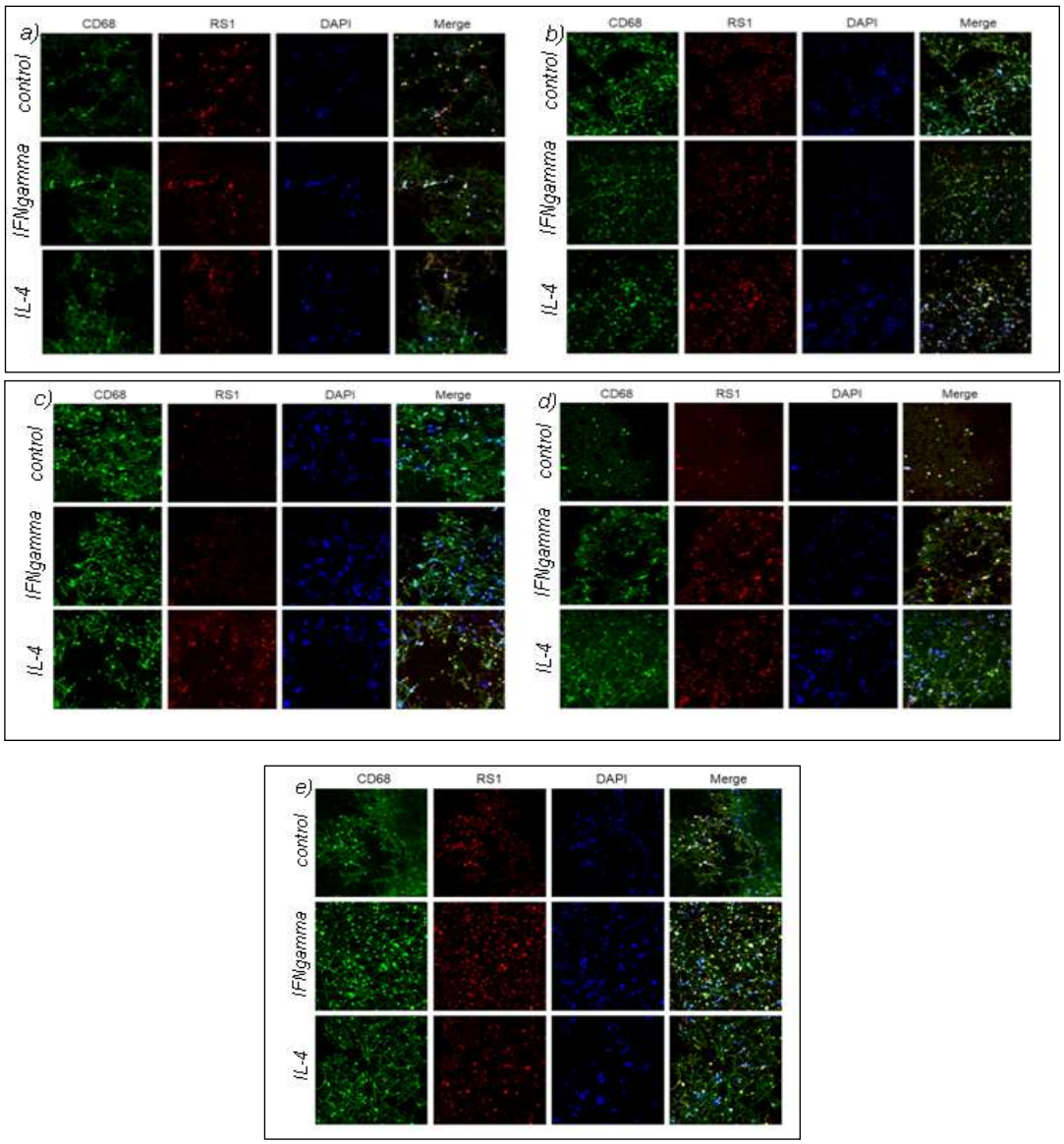

Figure 2. Analysis of CD68/RS1 phenotype macrophage on the sample surface: a – 1, b – 2, c – 3, d – 4, e – 5.

REFERENCES

1. *Kudryavtseva V., Stankevich K., Kibler E., Golovkin A., Mishanin A., Bolbasov E., Tverdokhlebov S.* The deposition of thin titanium-nitrogen coatings on the surface of PCL-based scaffolds for vascular tissue engineering. Applied Physics Letters. 2018; 112(15): 153705. doi: 10.1063/1.5017580.

2. *Gratchev A., Kzhyshkowska J., Köthe K., Muller-Molinet I., Kannookadan S., Utikal J., Goerdt S.* Mphi1 and Mphi2 can be re-polarized by Th2 or Th1 cytokines, respectively, and respond to exogenous danger signals. Immunobiology. 2006; 211(6-8): 473-86. doi: 10.1016/j.imbio.2006.05.017.