Applications of NSCLC Organoid Systems

Subjects: Cell Biology

Contributor: Raphael Werner

Lung cancer organoids hold the potential to be used for a variety of different translational research applications. A dynamic model system enables to simulate mechanisms that occur in vivo during cancer growth or under cancer treatment. In particular, the use as a platform for understanding tumor genomic evolution could be of interest, in order to elucidate how under the selective pressure of a given therapy resistance mechanisms develop. Being able to gain a deeper understanding of these processes might allow us to identify alternative treatment strategies for those patients developing resistance, e.g., to tyrosine kinase inhibitors (TKIs).

Keywords: non-small cell lung cancer ; organoids ; cancer model

1. Introduction

Lung cancer to date is the leading cause of cancer-related death worldwide, accounting for approximately 1.8 million deaths (18%), although in 2020 it was “only” the second most commonly diagnosed cancer (11.4%), having been marginally overtaken by breast cancer (11.7%) [1].

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases. In addition to the histopathological subclassification into adenocarcinomas (AC), squamous cell carcinomas (SCC) and large cell neuroendocrine carcinomas (LCNE), further subdivision based on molecular characteristics in recent years has been shown to be of additional importance as a number of the known oncogenic driver mutations in lung cancer are targetable. Unfortunately, in a significant number of patients with NSCLC, no targeted treatment options are available to date. A targeted treatment is only available in approximately 80% of all patients with lung AC and most commonly includes a treatment of sensitizing alterations in EGFR, ALK, ROS1, RET, MET or BRAF. Although 50% of SCCs present with an amplification in FGFR1 or an aberration in PI3K, neither are clinically targetable and the frequency of targetable driver mutations is so low that molecular testing is not routinely performed [2]. Consequently, it remains an urgent clinical need to identify additional therapeutic targets for those patients currently having to rely on standard chemotherapy but failing to respond, as well as second-line options for any targeted therapy patient developing resistance mechanisms.

While molecular characterization of the primary tumor is certainly crucial for the identification of potential novel therapeutic targets, it is equally important to be able to comprehensively evaluate any new therapeutic strategy in preclinical models. The first choice for the evaluation of novel therapeutic components is usually established commercially available cell lines grown in 2D. These are of course easily accessible and straightforward to handle, but also fundamentally flawed as they do not reflect and recapitulate the complex tumor heterogeneity of the primary tumor within the patient that will later be treated with the respective drugs. The complex composition of heterogenous tumor cells with different molecular phenotypes, together with the surrounding tumor microenvironment, is, however, what ultimately determines if and how a tumor responds to a given treatment. Application of complex, multicellular, three-dimensional organoid systems instead of standard 2D cell culture systems is therefore likely to provide a better assessment of the usefulness of a certain drug for the treatment of NSCLC in an early stage of preclinical assessment, and might even be able to avoid at least in part some of the preclinical animal testing.

2. Lung Cancer Organoids as Model Systems for Lung Cancer Biology Research

Traditional 2D cell culture models as well as transgenic mouse models are used to understand the underlying molecular mechanisms and pathway alterations leading to development and progression of cancer or to the development of drug resistance. Tumor organoids, as well as organoids generated from normal non-malignant cells, hold the potential to be used for the same purposes and to serve as ideal models for lung cancer biology research. Lo and colleagues, who have recently performed a comprehensive review of the available literature, have suggested organoids to be suitable for various cancer biology applications, including as a platform for functional genomics for oncogene discovery, to study

tumor genome evolution and cancer stem cells, as well as the involvement of oncogenic pathogens [3]. Lung cancer organoids hold the potential to be used for all of these described applications. In particular, the use as a platform for understanding tumor genomic evolution could be of particular interest, in order to elucidate how under the selective pressure of a given targeted therapy resistance mechanisms develop. Being able to gain a deeper understanding of these processes might allow us to identify alternative treatment strategies for those patients developing resistance, e.g., to tyrosine kinase inhibitors (TKIs).

3. Clinical Applications of NSCLC Organoid Systems: Present and Future

3.1. Assessment of Drug Sensitivity

Patient-derived NSCLC cell aggregates with spherical shape have already been used for chemosensitivity assays before the establishment of protocols that allowed long-term expansion of NSCLC organoids. After initial adherent culture, the group of Ruppen et al. used primary cells from three patients with NSCLC to form spherical aggregates by sedimentation in U-bottom microwells of a microfluidic device [4]. A subsequent cisplatin chemosensitivity assay in the microfluidic device demonstrated an increased chemoresistance when NSCLC cells were co-cultured with primary pericytes compared to a monoculture, suggesting a protective effect of pericytes for cancer cells [4].

To date, only few publications report the use of primary NSCLC organoids for drug screening. As an initial proof of concept, Endo et al. showed that growth of EGFR-mutated NSCLC organoids was suppressed by the EGFR TKIs erlotinib and gefitinib. These findings were further validated in PDX models [5]. Sachs et al. then demonstrated differential responses of NSCLC organoids to conventional chemotherapeutics such as cisplatin or paclitaxel, but also showed a sensitivity towards the TKIs such as erlotinib and gefitinib in ERBB2-mutant organoids [6]. In the study by Kim et al., a response to olaparib was seen in BRCA2-mutant NSCLC, a response to erlotinib in EGFR-mutant NSCLC and a response to crizotinib in EGFR-mutant and MET-amplified NSCLC organoids. The feasibility of a high-throughput drug response screening using 24 anti-cancer drugs including conventional chemotherapeutics and targeted treatments was demonstrated recently by Li et al. Notably, drug sensitivity remained consistent between different passages and drug responses correlated with the mutational profile of the parental NSCLC [7]. Shi et al. showed a strong synergetic effect using a combination treatment consisting of the MEK inhibitor trametinib and the FGFR inhibitor infigratinib in an organoid model of FGFR1 amplified lung squamous cell carcinoma [8]. Yokota et al. demonstrated that EGFR-TKI-resistant NSCLC organoids may respond to combination treatment of the Bcl-2 inhibitor navitoclax and the survivin inhibitor YM-155, and that BRAFG469A-mutated organoids were suppressed by a combination treatment of trametinib and erlotinib [9].

Despite these examples of successful drug screenings, a systematic clinical application of organoid models for personalized treatment decisions is not in sight. The time required to establish well-growing primary organoid cultures and the current rates of success and expansion limit the clinical application of organoids not only in NSCLC, but also in other cancer types such as colorectal cancer [10]. A promising solution for a faster and more straightforward drug screening is presented by Hu et al. [11] The group used early-passage (mostly p0) organoids in a microwell array chip for high-throughput analysis in a nanoliter scale and obtained drug response profiles within a week [11]. Being aware of the occurrence of genetic drift and a potential selection of rapidly growing and pluripotent cancer cells in advanced passages, the use of early-passage organoids may furthermore help to faithfully recapitulate the tumor heterogeneity and in vivo drug response and additionally fit the short time frame for an early clinical application after surgical resection [3]. Moreover, future organoid-based drug screenings should take into account biopsies from different tumor sites to help capture the parental tumor’s intratumoral heterogeneity more precisely [3].

3.2. Studying Cancer Stem Cells

The “cancer stem cell hypothesis” suggests that intratumoral heterogeneity is a result of the asymmetric cell division of a rare stem cell subpopulation with the capacity of self-renewal and pluripotency, which gives rise to a differentiated, but phenotypically diverse progeny [12]. In this sense, intratumoral heterogeneity and cancer stem cells are seen as the driving force behind minimal residual disease and resistance [12][13]. The investigation of cancer stem cells thus helps to understand NSCLC progression, and NSCLC stem cells are clinically promising targets for future treatment strategies. For this reason, the presence of cancer stem cell candidates in NSCLC organoids has been investigated in a small number of studies with diverging findings. Endo et al. reported no enrichment in CD133-positive cancer stem cell candidates among the organoids established in Matrigel and human embryonic stem cell culture medium. In contrast, Herreros-Pomares et al. reported an overexpression of genes related to stemness, namely p21, Notch3, CD44, integrin α6, Nanog and Snail, in lung adenocarcinoma organoids when compared to the adherently growing cells of the same tumor [14]. The group then generated a prognostic score based on the significantly overexpressed stemness genes to predict overall survival.

However, no significant difference in the expression of stemness-related genes was found in lung squamous cell carcinoma [14].

3.3. Whole-Organoid Xenografts

In the studies by Kim et al., Shi et al. and Herreros-Pomares et al., the tumorigenic potential of organoids was assessed by xenografting whole organoids in immunodeficient mice [15][14][8]. In the xenograft, the key biological and histological properties and the tumorigenicity of the parental tumor were preserved [8]. Upon transplanting NSCLC organoids, a faster growth and higher success arte in the establishment of the xenograft tumors was seen when compared to a parallel transplantation of dissociated cells into the same animal [15][14].

References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 2021, 71, 209–249.
2. König, D.; Prince, S.; Rothschild, S. Targeted Therapy in Advanced and Metastatic Non-Small Cell Lung Cancer. An Update on Treatment of the Most Important Actionable Oncogenic Driver Alterations. Cancers 2021, 13, 804.
3. Lo, Y.-H.; Karlsson, K.; Kuo, C.J. Applications of organoids for cancer biology and precision medicine. Nat. Rev. Cancer 2020, 1, 761–773.
4. Ruppen, J.; Wildhaber, F.D.; Strub, C.; Hall, S.R.R.; Schmid, R.A.; Geiser, T.; Guenat, O.T. Towards personalized medicine: Chemosensitivity assays of patient lung cancer cell spheroids in a perfused microfluidic platform. Lab Chip 2015, 15, 3076–3085.
5. Endo, H.; Okami, J.; Okuyama, H.; Kumagai, T.; Uchida, J.; Kondo, J.; Takehara, T.; Nishizawa, Y.; Imamura, F.; Higashiyama, M.; et al. Spheroid Culture of Primary Lung Cancer Cells with Neuregulin 1/HER3 Pathway Activation. J. Thorac. Oncol. 2013, 8, 131–139.
6. Sachs, N.; Papaspyropoulos, A.; Ommen, D.D.Z.; Heo, I.; Böttinger, L.; Klay, D.; Weeber, F.; Huelsz-Prince, G.; Iakobachvili, N.; Amatngalim, G.D.; et al. Long-term expanding human airway organoids for disease modeling. EMBO J. 2019, 38, e100300.
7. Li, Z.; Qian, Y.; Li, W.; Liu, L.; Yu, L.; Liu, X.; Wu, G.; Wang, Y.; Luo, W.; Fang, F.; et al. Human Lung Adenocarcinoma-Derived Organoid Models for Drug Screening. iScience 2020, 23, 101411.
8. Shi, R.; Radulovich, N.; Ng, C.; Liu, N.; Notsuda, H.; Cabanero, M.; Martins-Filho, S.N.; Raghavan, V.; Li, Q.; Mer, A.S.; et al. Organoid Cultures as Preclinical Models of Non–Small Cell Lung Cancer. Clin. Cancer Res. 2019, 26, 1162–1174.
9. Yokota, E.; Iwai, M.; Yukawa, T.; Yoshida, M.; Naomoto, Y.; Haisa, M.; Monobe, Y.; Takigawa, N.; Guo, M.; Maeda, Y.; et al. Clinical application of a lung cancer organoid (tumoroid) culture system. NPJ Precis. Oncol. 2021, 5, 29.
10. Ooft, S.N.; Weeber, F.; Dijkstra, K.K.; McLean, C.M.; Kaing, S.; van Werkhoven, E.; Schipper, L.; Hoes, L.; Vis, D.J.; van de Haar, J.; et al. Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients. Sci. Transl. Med. 2019, 11, eaay2574.
11. Hu, Y.; Sui, X.; Song, F.; Li, Y.; Li, K.; Chen, Z.; Yang, F.; Chen, X.; Zhang, Y.; Wang, X.; et al. Lung cancer organoids analyzed on microwell arrays predict drug responses of patients within a week. Nat. Commun. 2021, 12, 2581.
12. MacDonagh, L.; Gray, S.; Breen, E.; Cuffe, S.; Finn, S.; O'Byrne, K.J.; Barr, M.P. Lung cancer stem cells: The root of resistance. Cancer Lett. 2016, 372, 147–156.
13. Greaves, M. Evolutionary Determinants of Cancer. Cancer Discov. 2015, 5, 806–820.
14. Pomares, A.H.; De-Maya-Girones, J.D.; Calabuig-Fariñas, S.; Lucas-Dominguez, R.; Martínez, A.; Pardo-Sánchez, J.M.; Alonso, S.; Blasco, A.; Guijarro, R.; Martorell, M.; et al. Lung tumorspheres reveal cancer stem cell-like properties and a score with prognostic impact in resected non-small-cell lung cancer. Cell Death Dis. 2019, 10, 660.
15. Kim, M.; Mun, H.; Sung, C.O.; Cho, E.J.; Jeon, H.-J.; Chun, S.-M.; Jung, D.J.; Shin, T.H.; Jeong, G.S.; Kim, D.K.; et al. Patient-derived lung cancer organoids as in vitro cancer models for therapeutic screening. Nat. Commun. 2019, 10, 3991.