Gynaecological Malignancies

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Gynaecological malignancies represent a heterogeneous group of neoplasms with vastly different aetiology, risk factors, molecular drivers, and disease outcomes. From HPV-driven cervical cancer where early screening and molecular diagnostics efficiently reduced the number of advanced-stage diagnosis, prevalent and relatively well-treated endometrial cancers, to highly aggressive and mostly lethal high-grade serous ovarian cancer, malignancies of the female genital tract have unique presentations and distinct cell biology features. Recent discoveries of stem cell regulatory mechanisms, development of organoid cultures, and NGS analysis have provided valuable insights into the basic biology of these cancers that could help advance new-targeted therapeutic approaches.

Keywords: ovarian cancer ; endometrial cancer ; cervical cancer ; patient-derived organoids ; cancer stem cells ; Wnt signalling ; epithelial differentiation ; TP53 ; HDR mechanisms ; BRCA1/2

1. Introduction

Gynaecological malignancies represent a great medical burden for women's health in general. Together, uterine, cervical, and ovarian cancer are responsible for approximately 30,000 deaths every year in the USA (CDC data) [1], with an average of 10,000 deaths in Germany and similar prevalence also observed in other countries with well-organized health care systems. The incidence of cervical cancer continues to be disproportionally high in developing countries where early screening (Pap test), as well as HPV diagnostics and access to the vaccine, are not readily available. Endometrial cancer occurs more frequently than ovarian cancer, but a significantly shorter long-term survival in the latter case leads to disproportional high mortality, and ovarian cancer accounts for more than 50% of gynaecological cancer deaths [2]. While the overall difference in long-term prognosis is likely the consequence of earlier detection of endometrial cancer due to the early onset of more specific symptoms, substantial differences in the biology of these cancers and their responses to therapy are major factors that determine the course of the disease. The great diversity in molecular, histological, and genetic characteristics between ovarian, uterine, and cervical cancers is particularly intriguing due to all having the same developmental origins as all structures starting with the upper vagina, uterus and fallopian tubes develop from Mullerian ducts, as PAX8+ positive mucosal surfaces of columnar epithelium.

Although the whole genital system functions within the hormonal milieu during reproductive years, in some cases, the same physiological stimuli have opposing effects on cancer risk depending on the cancer type. For example, while oral contraceptives significantly reduce the risk of ovarian and endometrial cancers, they increase the risk for cervical cancer [3][4]. Moreover, obesity and diet are strongly associated with endometrial cancer risk [5] but appear not to play a major role in the development of high-grade serous ovarian cancer (HGSOC) [6]. These facts suggest that there are substantial differences in local homeostatic environments in areas of the genital tract and cancer type-specific combination of autonomous and exogenous factors are required to cause transformation.

2. Organoids Recapitulate the Main Characteristics and Tissue Hierarchy of Epithelial Tumours In Vitro, and Are a Potential Tool for Personalisation of Patient Therapy

Organoids are an attractive prospect for personalising patient treatments because it is now well established that patient-derived organoids (PDOs) resemble the parental tumour, as confirmed by genome sequencing and phenotypic analysis, and are applicable to drug screening [7]. Tumour ex vivo explant culture has also been established for different cancers and commercialized, with tailored TME ecosystems, to conserve patient/tumour heterogeneity and offer personalized therapeutic options for patients [8][9][10][11]. However, despite offering physiological, ethical, and financial advantages, explant cultures have a very short window of opportunity for therapeutic screening. In contrast, PDOs have multiple advantages such as self-renewal and expansion to test multiple drugs/combinations, capable of long-term storage for bio-banking and future regeneration for drug screening, and suitability for molecular genetic manipulation. Analogous to

organoid formation capacity and in vitro differentiation potential of stem cells from the healthy epithelium from the fallopian tube, endometrial glands, and cervix epithelium, malignant tumours of the genital tract also contain stemness potential that drives developments of cancer organoids in vitro. Longevity of stem cells which drive expansion of organoids in culture and continuous differentiation make organoids adequate models to test in vitro both strategies that have been previously described as viable approaches to eradicate CSCs [12], by direct targeting of sternness potential and forced differentiation.

A recent study establishing HGSOC PDOs from chemo-naïve tumours identified a shift in required stem cell growth conditions for HGSOC, in contrast to healthy Fallopian tube organoids, and detected that key regulatory changes in markers of stemness and differentiation occur early in the development of HGSOC tumourigenesis [13]. However, in advanced HGSOC, evolution in the cellular mechanisms which preserve CSCs potency remains elusive, and our knowledge about molecular origins of chemoresistant clones is rudimentary. Little is known about the clonal evolution of CSCs from primary disseminated disease to recurrent HGSOC, the presence of CSC populations within relapse tumours, and how they relate to the initial stem cell population in the primary tumour.

Propagation of PDOs to determine drug or radiotherapy efficacy is becoming more commonplace for different gynaecological malignancies. In particular, significant progress has been made for establishing drug treatment parameters and platforms for PDOs derived from EOC tumours, albeit currently at a low-medium throughput level. PDOs from EOC cases were treated with standard-of-care first-line chemotherapies (carboplatin, cisplatin, paclitaxel) and relevant targeted therapeutic agents (PARP inhibitors, PI3K inhibitors), and responses recorded correlated with the patient's clinical responses [7][14][15][16]. A direct comparison of drug screening in 2D ovarian tumour cells and ovarian cancer PDOs demonstrated that cytostatic drug efficacy differs between the two culture systems, linking organoid drug sensitivity to DNA repair deficiency in the PDOs, findings not noted in the 2D monolayer cultures [16]. Moreover, PDOs revealed inter- and intra-patient heterogeneity in responses to drug treatments for a small number of patients [7][14], strongly illustrating that subsequent studies must include further sites of tumour dissemination to fully characterise the intrinsic heterogeneity existing within patients with EOC and accurately model tumour therapeutic responses. A potential limiting factor of a number of previous studies on EOC organoids is the propagation of organoids from neo-adjuvant tumours [7][14]; these tumour samples may already exhibit biological variations due to pre-treatment with chemotherapy [13].

Studies which established human endometrial PDOs include the optimization and differentiation of hormone-responsive organoid cultures. Furthermore, upon exposure to pregnancy signals, endometrial organoids displayed characteristics of early pregnancy [17]. Organoids mimicked the normal physiological responses of the endometrium to hormonal control, e.g., oestrogen promoted increased cell proliferation, and additionally, hormone treatment of human endometrial organoids allowed replication of the menstrual cycle [18]. Single-cell RNA-sequencing was employed to create a high-resolution gene expression atlas of endometrium organoids, and provided information on their responsiveness to hormone treatment (oestrogen and progesterone), replicating gene expression changes in proliferative and secretory phase endometrium [19]. PDOs have also been established from endometriosis, precancerous states such as endometrial hyperplasia and Lynch syndrome as well as low and high-grade endometrial cancers [20]. PDOs from these endometrial diseases demonstrated long-term expansion properties, transcriptomic and genomic stability, and captured the clinical heterogeneity of each condition and disease setting. Endometrial cancer organoids were amenable to drug screening of standard chemotherapies, demonstrating patient-specific drug responses, in particular sensitivity to mTOR inhibition in line with mutations in the PI3K/AKT/mTOR signalling pathway [20].

Recent comprehensive studies deriving PDOs from cervical cancer cells or normal cervical tissue have been described. Organoids have been developed from normal cervical cells from the SCJ region, and metastatic squamous cells from the transformation zone, providing models in which HPV-driven cervical carcinogenesis could be evaluated [21]. Furthermore, organoids established from human and mouse ecto- and endo-cervical cells revealed that the two epithelial cell types descend from distinct cervical lineage-specific stem cell populations which are regulated by divergent stromal Wnt signals. PDOs from human ecto- and endo-cervical lineages faithfully recapitulated the in vivo architecture and could be maintained in culture for more than six months [22]. Radiation therapy is a mainstay therapy for cervical cancer patients, and the radiosensitivity of cervical cancers is quite diverse. Radiotherapy treatment of organoids could be an alternative approach to predict radiation sensitivity of patient tumours. Different groups have attempted to interrogate the radiosensitivity of cervical tumour organoids and spheroids to investigate mechanisms underlying radiation resistance. Inhibition of growth of small cell carcinoma of the uterine cervix (SCCC) organoids was observed in a dose-dependent manner post-irradiation, and variable radiosensitivity profiles were detected across PDOs from individual patients. Radiation-induced upregulation of HIF-1α in radioresistant SCC organoids was proposed as a mechanism of radioresistance [23]. Organoids derived from another rare subtype of cervical cancer, clear cell cervical cancer (cCCC),

were found to resemble the tumour of origin and demonstrated chemosensitivity following treatment with common chemotherapy agents and MET inhibitors [24].

In addition to a necessary focus on cancer driving genes and developmental pathways which control epithelial homeostasis, it is becoming clear that micro-RNAs and noncoding RNAs could regulate CSC potential on a post-transcriptional level. Micro-RNA family miR-34 has been described in several malignancies as tumour-suppressive and thus could be a promising pharmacological therapeutic candidate [25]. As TP53 is the main regulator of miR-34 expression, this could be of great interest for HGSOC treatment [26]. Indeed, a recent study found significantly lower levels of miR-34 in HGSOC tumours in comparison to low-grade ovarian cancer tissue, in line with differences in TP53 mutation status [27].

3. Conclusions

Clinical Perspective

Taken together, recent advances in our understanding of changes in stem cell regulation and epithelial homeostasis during carcinogenesis and disease progression have opened promising new approaches in the research of gynaecological malignancies, with great translational potential. Cancer stem cells appear be able to modulate core signalling pathways in epithelial ovarian cancer and are believed to be responsible for disease progression, relapse, and drug resistance development [28]. Hence, cancer stem cells appear to play a key role not only in carcinogenesis but also in the natural history of the disease and contribute to its heterogeneous profile. They represent a promising therapeutic platform that, once decoded, can potentially open numerous possibilities for the resolution of this challenging cancer entity [1]. Evidence has demonstrated cancer stem cells induce and influence progression or relapse and are characterized by a rather slow-cycling rate which makes them resistant to standard cytotoxic treatments [28][29][30][31]. Presumed hypotheses suggest that the high probability of relapse of advanced epithelial ovarian cancer is possibly attributed to a subpopulation of quiescent epithelial ovarian cancer stem cells that, by remaining in the G0 phase of the cell cycle, are not sensitive to cytotoxic treatments. However, once they return to an active reproduction phase, they can become the potential driving force of the cancer relapse [28]. Direct correlations between the onset of chemoresistance and the abundance of CSCs is an emerging field and requires further exploration and validation [31][32][33]. In addition, long-term follow-up and functional analysis of PDOs derived from patients at different stages of disease progression could help identify mechanisms of cellular perturbations that drive tumour growth in response to therapy.

A further perspective for exploration is the field of early diagnosis and detection of CSCs. Many recent technologies have focused on the endoscopic or hysteroscopic examination of the fallopian tubes and/or uterine lavage fluid with clinical trials ongoing to test these devices [34]. These technologies are based on the principle that cells from HGSOC, or precursor STIC lesions, can exfoliate and be secreted together with the endometrial fluid, which in turn can be easily accessed, representing a promising avenue for earlier diagnosis [34]. Although currently, collection of these cells is challenging and not yet routine, it is a highly promising emerging approach that could be implemented into clinical practice once the technology is fully optimised. We could envisage the development of organoids from the cells isolated from uterine lavage that can in turn be used for profiling the biology of the disease and to predict patients' response to treatment.

Overall, it can be concluded that complex 3D patient-derived organoid culture models provide new and versatile experimental platforms to study gynaecological malignancies. They create opportunities to identify central mechanisms of cancer biology, which remain poorly understood such as tissue hierarchy and clonal diversification, influence of microenvironment and interactions with immune system. This could lead to development of new, better-tailored therapeutic concepts that help towards achieving the long-term goal of improving detection and patient outcomes.

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