Tumour Heterogeneity in Gastroenteropancreatic-Neoplasms

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Tumour heterogeneity is a common phenomenon in gastroenteropancreatic (GEP)-neoplasms (NENs) and has a negative impact on treatment success and prognosis as it produces cell clones that do not express treatment targets (i.e., SSTR, mammalian target of rapamycin–mTOR- signalling pathway, Ki-67).

neuroendocrine tumour | neuroendocrine neoplasms | gastroenteropancreatic

1. Introduction

Tumour heterogeneity refers to spatial and temporal variations that may occur within the tumour environment (intra-tumour) or within individual tumour foci, and also between tumour sites (inter-tumour) [1]. Such heterogeneity may encompass genetic and epigenetic variations, or differences in the tumour microenvironment [2][3][4][5][6]. Tumour heterogeneity can also evolve over time due to selective pressures, such as those imposed by treatment, leading to selection and clonal expansion of subpopulations [1][7][8][9]. Tumour heterogeneity is common in human tumours and its occurrence is essential to understand and predict tumour progression and response to specific treatment [10]. Higher intra-tumour and/or inter-tumour heterogeneity can be associated with negative outcomes [7].

Neuroendocrine tumours (NETs), better defined as neoplasms (NENs), are a heterogeneous group of neoplasms that range from well-differentiated tumours to more aggressive carcinomas (**Table 1**) [11].

Table 1. Classification for gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs).

Terminology	Differentiation	Grade	Mitotic Rate (Mitoses/2 mm^2) *	Ki-67 Index % **
NET, G1	Well differentiated	Low	<2	<3%
NET, G2		Intermediate	2–20	3–20%
NET, G3		High	>20	>20%
NEC, small-cell type (SCNEC)	Poorly differentiated	High	>20	>20%
NEC, large-cell type (LCNEC)			>20	>20%

Terminology	Differentiation	Grade	Mitotic Rate (Mitoses/2 mm^2) *	Ki-67 Index % **
MiNEN	Well or poorly differentiated	Variable	Variable	Variable

* mitotic rates are determined by counting in 50 fields of 0.2 mm^2; ** Ki-67 values are expressed as the percentage of positive cells. Abbreviations: LCNEC, large cell neuroendocrine carcinoma; MiNEN, mixed neuroendocrine–non-neuroendocrine neoplasm; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumour; SCNEC, small cell neuroendocrine carcinoma.

ell growth and hormone secretion in normal and cancerous neuroendocrine cells [12]. Somatostatin receptors are G-protein-coupled receptors with a typical transmembrane domain that includes five distinct subtypes named 1 to 5, with the gene encoding for the SSTR2 also producing two splice variants, SSTR2 isoform A and B. While the natural ligands of SSTRs (i.e., somatostatin-14, somatostatin-28 and cortistatin) all bind to the receptors with high affinity, somatostatin analogues (SSAs)-octreotide, vapreotide and lanreotide-bind only to SSTR2 and with a lower affinity to SSTR3 and 5 [13][14][15]. Neuroendocrine neoplasms express all SSTRs at different concentrations, with SSTR2 being the predominant receptor found across NENs of different origins, followed by SSTR3 in gastroenteropancreatic (GEP)-NENs and SSTR1 and SSTR5 in midgut NENs [16][17][18].

The GEP tract is the most common site for NENs, with the small intestine (SI) and the pancreas being the most prevalent sites of origin for more advanced neoplasms. For these neoplasms, treatment strategies are based on information on SSTRs expression, tumour stage and grade (including differentiation) and the expression of neuroendocrine biomarkers [19].

The definitive diagnosis of an NEN is made by histopathological examination of tumour tissue, obtained either via a biopsy or following surgery. Morphologic imaging, however, is essential as a baseline evaluation for staging, in particular for identifying the presence of metastases, while functional imaging is important to assess the functional and metabolic status of the tumour. Combining morphological (e.g., computer tomography-CT) and functional imaging techniques is fundamental in the decision-making process of the therapeutic approach to patients with GEP-NENs [20]. Gallium68 (68Ga)-DOTA-peptide positron emission tomography (PET)/CT, i.e., 68Ga-DOTATATE or 68Ga-DOTATOC, remains the gold standard for assessing the eligibility and response to peptide receptor radionuclide therapy (PRRT), especially for well-differentiated grade 1 and grade 2 GEP-NETs [21][22]. However, NENs often show heterogeneous expression of SSTR, which could lead to inferior outcomes following targeted treatment and subsequently influence relapse and progression of the disease [21][22][23][24][25]. High-grade lesions and metastases can have a lower expression of SSTRs which may not be fully assessed on receptor-based imaging alone.

Spatial and temporal heterogeneity should be taken into account in the assessment of NENs, as it is not uncommon for GEP primary and metastatic sites to show intra-tumour and inter-tumour heterogeneity in their Ki-67 index, as well as in their SSTR expression and cell signalling pathways, leading to incomplete understanding of their tumour biology and behaviour [26][27][28][29][30][31].

2. Tumour Heterogeneity in GEP-NENs

Tumour heterogeneity is a common phenomenon in GEP-NENs (**Figure 1**) and has a negative impact on treatment success and prognosis as it produces cell clones that do not express treatment targets (i.e., SSTR, mammalian

target of rapamycin–mTOR- signalling pathway, Ki-67) [32].

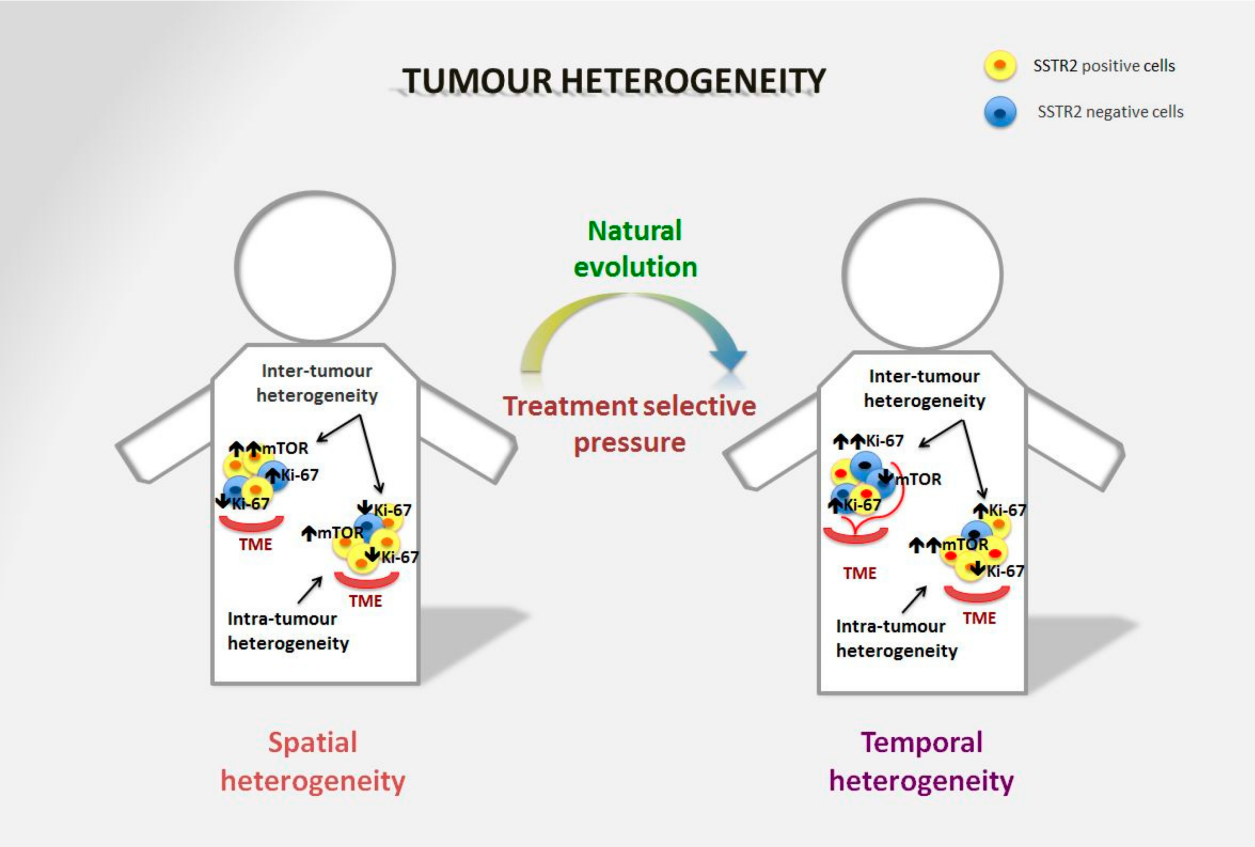

Figure 1. Spatial and temporal heterogeneity in NENs. Neuroendocrine neoplasms generally express SSTR2 on the tumour surface, and are well-differentiated tumours in the majority of cases. However, spatial heterogeneity within the primary tumour may lead to the presence of areas with lower expression of SSTR2 and/or a different Ki-67 index. This heterogeneity is also frequent in metastatic sites and can differ significantly from the primary lesion. The mTOR pathway is also commonly involved in the onset of the disease and is particularly relevant in distant metastases, although over time alternative pathways may be involved in tumour survival. Moreover, temporal heterogeneity that can be linked to treatment selective pressure may lead to significant changes in tumour biology that affect prognosis and survival.

Pancreatic NENs can show a progressive increase in their Ki-67 index or progression to a more aggressive disease, events that are linked to poorer prognosis [33][34]. Changes in the intra-tumoural distribution of Ki-67 in GEP-NENs can lead to significant downgrading of tumours as a consequence of sample bias, especially when small samples are collected that include areas of non-neoplastic tissue [31][35][36]. The Ki-67 is one of the prognostic markers for NENs; however, evaluation of the Ki-67 depends on the site and size of the tumour biopsy and assessment by the pathologist, therefore it may be not representative of tumour behaviour in heterogeneous lesions and especially in intermediate grade 2 lesions [26].

Small-intestine NENs are generally considered to have a relatively low somatic mutation rate, but a more florid epigenetic derangement. It has been shown, however, that there is a high degree of genetic variability between the

primary site and liver metastases [37]. Although they are generally well-differentiated tumours with low proliferation rates, distant metastases, in particular hepatic, are a common event and an important cause of poor prognosis [38][39]. The rate of mutations is high, especially in liver metastases, with the mutations often being different to the mutations seen within the primary tumour, thus demonstrating a unique pattern of metastatic spread of SI-NENs [37][40][41]. A large molecular profiling study on SI neuroendocrine liver metastases showed that the expression of several cancer-related pathways that promote tumour development, progression and angiogenesis, including phosphoinositide 3 kinase (PI3K), epidermal growth factor receptor (ErbB1), platelet-derived growth factor receptor beta (PDGFRβ) and mTOR, is upregulated in neuroendocrine liver metastases in comparison to their primary site, and that neuroendocrine liver metastases harbour progressive genomic aberrations that occur mostly during the metastatic progression of the tumour [42]. It has also been shown that the pattern of metastatic growth within the liver may be the expression of the different biological behaviour of the disease, as less-differentiated NENs more often showed an aggressive pattern of growth (disseminated metastatic spread) linked to higher Ki-67 and more advanced disease [43]. Most metastases of GEP-NENs show a higher Ki-67 proliferation index than the primary tumour site, meaning that metastatic spread is potentially unrelated to its initial phenotype or genotype [26][44][45]. SI-NENs are usually believed to display an even expression of SSTR2 isoform A [24]. However, SI neuroendocrine liver metastases often show heterogeneous SSTR2 isoform A expression between lesions in the same patient, this seems to be unrelated to the tumour proliferation index or the tumour size, confirming that expression in metastatic lesions is not always similar to that in the primary tumour or between lesions in the same patient [24][31]. Moreover, no correlation has been shown with the SSTR2 isoform A expression of the primary tumours [24]. Liver metastases from ileal NENs have also been found to show a higher expression of SSTR5, which is potentially linked to tumour aggressiveness [46]. Somatostatin receptor type 5 expression seems to correlate with the presence of metastases and angioinvasion in NENs [47]. Although imaging, in particular 68Ga-DOTATATE PET/CT scans, seems to detect most liver metastases even when SSTRs expression is weak, response to radioligand therapy (RLT) may be lower and different between lesions in the same patient [24][48]. Intra-tumour, and especially inter-tumour, heterogeneity should therefore be taken into account in the diagnosis and management of GEP-NENs, as it represents a major challenge for the efficacy of targeted therapies. A better understanding of tumour biology will help in maximizing treatment outcomes.

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