Breast Cancer: Targets and Therapy Dovepress

Open Access Full Text Article REVIEW

Exploring the Complexity of Pan-Cancer: Gene Convergences and in silico Analyses

Leandro Teodoro[1,2], Ana Claudia O Carreira[1,3], Mari C Sogayar[1,2]

[1]Cell and Molecular Therapy NUCEL Group, School of Medicine, University of São Paulo, São Paulo, São Paulo, 01246-903, Brazil; [2]Biochemistry Department, Chemistry Institute, University of São Paulo, São Paulo, São Paulo, 05508-900, Brazil; [3]Center of Human and Natural Sciences, Federal University of ABC, Santo André, São Paulo, 09280-560, Brazil

Correspondence: Mari C Sogayar, Institute of Chemistry – University of São Paulo, Prof. Lineu Prestes Avenue, 748 - Cidade Universitária, São Paulo, São Paulo, 05508-000, Brazil, Tel +5511992042844, Email mcsoga@iq.usp.br

Abstract: Cancer is a complex and multifaceted group of diseases characterized by highly intricate mechanisms of tumorigenesis and tumor progression, which complicates diagnosis, prognosis, and treatment. In recent years, targeted therapies have gained prominence by focusing on specific mutations and molecular features unique to each tumor type, offering more effective and personalized treatment options. However, it is equally critical to explore the genetic commonalities across different types of cancer, which has led to the rise of pan-cancer studies. These approaches help identify shared therapeutic targets across various tumor types, enabling the development of broader and potentially more widely applicable treatment strategies. This review aims to provide a comprehensive overview of key concepts related to tumors, including tumorigenesis processes, the tumor microenvironment, and the role of extracellular vesicles in tumor biology. Additionally, we explore the molecular interactions and mechanisms driving tumor progression, with a particular focus on the pan-cancer perspective. To achieve this, we conducted an in silico analysis using publicly available datasets, which facilitated the identification of both common and divergent genetic and molecular patterns across different tumor types. By integrating these diverse areas, this review offers a clearer and deeper understanding of the factors influencing tumorigenesis and highlights potential therapeutic targets.

Keywords: pan-cancer, tumor therapy, tumor biomarkers

Introduction

Cancer may be defined as a set of multifaceted diseases that converge through deregulation of both replicative processes, leading to abnormal cell proliferation, and of molecular mechanisms linked to cell death.

Tumorigenesis primarily occurs due to factors such as mutations in key genes (oncogenes and/or tumor suppressor genes), epithelial–mesenchymal transition process (EMT), emergence of a tumor microenvironment favoring tumor progression, and activation of immune evasion mechanisms, angiogenesis, and metastasis.

In 2020, the global incidence of cancer exceeded 19 million cases, with breast, lung, and colorectal cancers having the highest absolute number of cases, regardless of sex or age, respectively.[1] In the same year, the global mortality rate, regardless of sex or age, reached 51.61%, with lung, colorectal, and liver tumors representing 35.7% of this total.[1]

Tumor Classification

Tumors can be categorized as in situ or invasive, corresponding to the so-called benign and malignant cancers, respectively. Tumor classification can also utilize anatomical characteristics, according to the Classification of Malignant Tumors (TNM), which uses tumor extent based on the extent of the primary tumor (T, T1 – T4); the absence or presence of metastasis in adjacent lymph nodes (N, N0 – N2), and the absence or presence of distant metastasis from the primary site (M, M0 – M1). The TNM varies depending on the type of tumor, as exemplified in Table 1 below:

Received: 5 September 2024
Accepted: 6 November 2024
Published: 13 December 2024

Table 1 TNM Classification of Different Tumor Types

Tumor extension (T)	**T1 (cm)**	**T2 (cm)**	**T3 (cm)**	**T4 (cm)**
Head and Neck	≤ 2	2 ≤ *x* < 4	≥ 4	Moderate metastatic progression
Gastrointestinal	2 ≤ *x* < 5	5 ≤ *x* < 10	≥ 10	
Skin	≤ 2	2 ≤ *x* < 4	≥ 4	Initial invasion
Urinary	≤ 2	2 ≤ *x* < 4	≥ 4	
Pulmonary	< 3	3 ≤ *x* < 5	5 ≤ *x* < 7	≥ 7
Metastasis in Adjacent Lymph Nodes (N)	**N0**	**N1**	**N2**	
Head and Neck	No metastasis to nearby lymph nodes	1 lymph node affected	2 or more lymph nodes affected	
Gastrointestinal				
Skin				
Urinary				
Pulmonary				
Distant Metastasis (M)	**M0**		**M1**	
All tumors	Absence		Presence	

Biomarkers

Although histopathological analyses are important in oncological research, biomarkers have increasingly become the main focus in tumor diagnosis and clinical prognosis. Biomarkers are generally associated with cellular processes broadly related to tumorigenesis and tumor progression, such as uncontrolled proliferation, differentiation and cell migration, in addition to being involved in the deregulation of molecular functions associated with cell adhesion, cell communication and invasiveness[2] (Table 2).

Table 2 Main Tumor Biomarkers Used in Clinic

Biomarker	**Structure and/or Function**	**Related Tumor**
α-Fetoprotein	Fetal Serum Protein: plasmatic transport	Gastrointestinal
BTA	Bladder Tumor Antigen	Urothelial
Telomerase	Ribonucleoprotein: telomeric region mantaining	Bladder
P53	Tumor-suppressor protein p53: Cell Cycle control	Many
Ki-67	Cell Cycle control	Breast
MCA	Glycoprotein	Reproductive; Breast
Cyfra 21.1	Cytokeratins-derived antigen	Head/Neck; Lung
PAP	Acid Phosphatase	Prostate
CA 15.3	Immune-associated Glycoprotein	Breast
CA 19.9	O-glycan-linked tetrasaccharide associated with cell-cell recognition	Pancreatic; Colorectal

(*Continued*)

Table 2 (Continued).

Biomarker	Structure and/or Function	Related Tumor
Catepsin D	Protease	Lymph Nodes metastasis
K-Ras	Protein linked to proliferation processes	Many
PSA	Serine-protease	Prostate

Tumor Microenvironment

Recently, the tumor microenvironment has become a major player in tumor studies, regulating neoplastic progression processes,[3] with direct action in tumorigenesis and cell migration exerted by fibroblasts, endothelial cells, and adipocytes. These components also act through molecular factors such as Vascular Endothelial Growth Factor (VEGF), in tumor neovascularization and metabolism.[4] Non-cellular components also directly contribute to the progression and increase in tumor malignancy, with the extracellular matrix (ECM) being the primary component. Composed of fibronectin, collagen, elastin, and laminin, the ECM provides a physical network that forms the main structure of solid tumors.[5] The process of desmoplasia, in which connective tissue, richly composed of ECM, grows abnormally adjacent to a preexisting tumor, is directly associated with a poor prognosis for patients.[6]

In summary, tumors, regardless of their classification, exhibit a high degree of heterogeneity at their core, relating to the types of genes expressed, associated biomarkers, existing regulatory pathways, and the tumor microenvironment, which varies in components and degrees of differentiation, depending on the tumor in question. However, despite their heterogeneity, tumors converge at various points, from their origin to the stages of neoplastic progression. A new area of study has emerged in tumor research, utilizing contemporary biocomputational tools to correlate hundreds of tumor subtypes and their convergent processes.

Pan-Cancer

Pan-cancer is a new concept aimed at studying the heterogeneity of tumors from a macro perspective, utilizing next-generation sequencing technologies capable of generating extensive databases. By integrating biocomputational technologies, these databases comparatively examine the similarities and differences among various types of cancers, thus facilitating the integration of migration, proliferation, and tumor invasion within a general genetic regulatory network framework.[7]

Biobanks have led to a substantial increase in the number of potential genes that may be related to tumorigenic processes, especially from similar genetic signatures across different tumor types.[8] Such signatures may be associated with gene substitutions, insertions, or deletions, as shown in Table 3.

Table 3 Genetic Mutations Related to Tumors

Signatures	Etiology	Related Tumors
Single base substitutions (SBS)	Deamination of 5-methylcytosine	Adenocarcinoma
		Breast
		Colorectal
		Ovarian
		Stomach
		Uterine

(*Continued*)

Table 3 (Continued).

Signatures	Etiology	Related Tumors
Doublet base substitutions (DBS)	Nicotine, pollution	Liver
		Lung
		Biliary Adenocarcinoma
		Bladder
		Stomach
Insertions/Deletions (Indels)	Dysfunctions in the DNA mismatch repair mechanism	Adenocarcinoma
		Breast
		Colorectal
		Ovarian
		Stomach
		Uterine
		Head and Neck
		Melanoma
		Endocrine
		Lymphoma

In addition to the aforementioned genetic signatures, international consortia propose using many omics data as a method to search for patterns of cellular origin (cell-of-origin) based on analyses of molecular similarities, which relate the origins of tumor cells with the aspects of in situ and invasive tumors; oncogenic processes, which analyze how genetic variants and somatic mutations influence tumor progression; and, also, standard mechanisms associated with key molecular pathways correlated with genes previously established to act in tumor malignancy (MYC, RAS) and cellular processes directed toward ubiquitination, splicing, and energy metabolism.[9]

The goal is to analyze the state of the art concerning tumors, based on the refinement and analysis of public data linked to multi-omics assays in pan-cancer, seeking to identify convergent and divergent patterns concerning gene mutations and differential expression of protein-coding genes, aiming to establish potential new proteins that may be associated with the processes of tumorigenesis and tumor progression.

Methods

A literature search was conducted on PubMed and Google Scholar databases for the last 10 years. We selected 101 articles/public data repositories correlated to basic and clinical research on numerous tumor-related topics: history, epidemiology, intratumoral environment, tumor microenvironment, tumor evasion, tumorigenesis, tumor progression, tumor therapeutics, tumor biomarkers, pan-cancer, tumor cell-of-origin, and extracellular vesicles in the tumor context.

For multi-omics analyses, the following public repositories were used: Pan-cancer Analysis of Whole Genomes of International Cancer Genome Consortium/The Cancer Genome Atlas Program (ICGC/TCGA), China Pan-cancer, and Metastatic Solid Cancers. These repositories provided epidemiological, genetic, and protein prediction information for 20 tumor types. The analyses were conducted using the cBioPortal omics refinement and analysis suite, along with EnrichR for Gene Set Enrichment Analysis and StringDB for protein interaction searches.

Tumor Onset

The current state-of-The-art in tumorigenesis includes the following elements and genes, which are common to all tumors (Figure 1):

- Sequential mutations, which can arise from direct genomic alterations or epigenetic modifications, contributing to a disrupted homeostatic environment and uncontrolled cell proliferation;[10]
- Loss of communication with neighboring cells, enabling evasion of the immune system;[11]
- Ability to express cell survival-promoting factors (or cell immortality through telomerase activation in tumors) and inhibit apoptotic pathways (eg, caspase cascade);[10]
- Disruption of normal metabolic pathways that typically involve diverting carbon sources from healthy tissues, increased aerobic glycolysis with elevated lactate production, and a super-stimulus for VEGF-driven angiogenesis;[12,13]
- Promotion of inflammatory processes through immune cell recruitment or evasion;[14]
- Establishment of invasive processes leading to lymph node and distant metastases.

Based on these aspects, cancer can be defined as a complex system of diseases caused by various factors, from deregulation of the mitotic cycle, considering all its inherent complexity (eg, deregulation of proteins from interphase or anaphase checkpoints),[15,16] accumulation of mutations in different genomic regions that cause unavoidable instabilities in the affected cells, to unique mutations in key genes linked to the processes of proliferation, adhesion, and cell migration, such as those encoding p53, CDK1, KIP, BRCA, among others.[17]

Another hypothesis proposes that tumor stem cells (TSCs) drive tumorigenesis. Due to their similar characteristics, when compared to normal stem cells, TSCs sustain tumor growth and contribute to resistance against conventional therapies, such as radiotherapy and chemotherapy.[18]

Nevertheless, despite their shared origins, tumors exhibit significant heterogeneity, influenced by genetic mutations, the tumor microenvironment, epigenetic factors, and complex molecular mechanisms. Consequently, diagnosing, prognosing, treating, and understanding the global epidemiology of cancer remains a formidable challenge.

Data from the TCGA Project, considering the ratio of synonymous to non-synonymous mutations in human populations, suggest that early-stage cancer on a microevolutionary scale is a neutral process for species fitness. This is indicated by a ratio close to 1 for various tumor-associated genes, contrary to the belief that this ratio would be significantly higher for tumors. However, key genes present extremely high values of this ratio, further characterizing them as a focus of studies in the field of pan-cancer.[19]

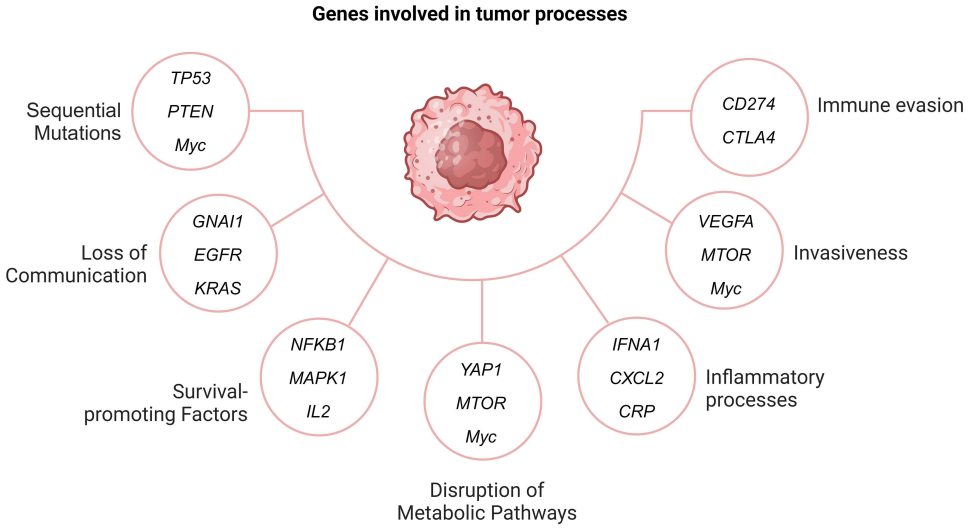

Figure 1 Genes involved in tumor processes. Main processes related to tumorigenesis and tumor progression and their main mutated genes.

Thus, an environment of high genetic instability is created, which, coupled with other conditions (altered metabolism, support from tumor-associated cells present in the tumor microenvironment), facilitates tumorigenesis and tumor progression, guided by a microevolutionary sense of selective adaptation of normal cells.

Tumor Morphological Changes

Cell differentiation is essential for creating specialized cells with distinct functions, yet it is not the sole factor driving the complexity of organisms. EMT is a process of transforming polarized epithelial cells into mesenchymal cells. It is essential for neural crest gastrulation and delamination, as well as the generation of motile cells capable of degrading the tissues basal membranes.[20] However, due to this characteristic, EMT is strongly associated with tumorigenesis and tumor progression. During EMT, both normal and cancer cells undergo profound physiological changes, driven by transcription factors such as TWIST1/2, Snail, Slug, and ZEB1/2, regulating the expression of cell junction proteins, such as cadherins.[21]

E-cadherin and N-cadherin are responsible for epithelial cell adhesion, signaling, adherent junction formation, and migration. These proteins play essential roles in maintaining cell structure and communication in different tissue types. However, in tumorigenic environments, these transcription factors and proteins facilitate EMT by suppressing E-cadherin expression and upregulating N-cadherin. E-cadherin suppression is primarily caused by the silencing of the CDH1 promoter in E-box regions by Snail, Slug, and Twist,[22,23] which allows cells to lose their adherence to one another and acquire a more mesenchymal property. N-cadherin activation is influenced by factors linked to E-cadherin, such as Twist and the methyltransferase KMT5A. These proteins interact to induce N-cadherin promoter region through H4K20 methylation,[24] facilitating the cell transition to a mesenchymal phenotype, thereby promoting migration and invasion.[25]

Classical Tumorigenesis-Associated Molecular Pathways

Tumorigenesis-associated molecular pathways are essential for understanding tumor initiation and progression. These intricate cell communication networks regulate normal cell growth, proliferation, survival, and differentiation, but their subversion drives cancer onset and progression.[26]

Extracellular-Regulated Kinase/Mitogen-Activated Protein Kinase Pathway

The extracellular-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) pathway is central to regulating cell proliferation, differentiation, apoptosis, and cell stress responses.[27] Its activation occurs mainly through binding of s and peptide growth/differentiation factors to plasma membrane tyrosine kinase receptors (TKRs). This engagement triggers the signaling pathway by activating small GTPases, such as the Ras protein, which then uses a second messenger (G protein) to activate Raf proteins. These, in turn, initiate a phosphorylation cascade, first of MEK1/2, at their serine residues, and then of ERK1/2 through direct action of MEK1/2, facilitating translocation of ERK1/2 to the nucleus.[28]

ERK1/2 translocation to the nucleus carries out various effector actions linked to cell proliferation, migration, and differentiation, such as phosphorylation of transcription factors c-Jun, c-Fos, and c-Myc, regulation of ribosomal kinases, negative feedback of factors linked to the pathway itself, as well as induction of gene coding for proteins associated with EMT, the cytoskeleton, and the cell cycle.[29]

Phosphatidylinositol 3-Kinase/Protein Kinase B Pathway

The phosphatidylinositol-3-kinase (PI3K/Akt) signaling pathway is a vital player in intracellular signaling, influencing not only cell proliferation but also the regulation of cell survival, stress response, growth, and metabolism, as well as acting as an effector in cytoskeletal reorganization under various conditions.[30] Numerous oncogenes and peptide growth factors are associated with its activation, which is also directly modulated by epigenomic modifications.[31] In tumors, its activation occurs through two primary mechanisms linked to tyrosine kinase receptor activation, while its overactivation is associated with PTEN tumor suppressor gene inhibition.[32,33] Several stages are involved in PI3K/Akt pathway activation. Peptide growth factor stimuli activate PIK protein subunits through PIP3 synthesis by phosphorylating PIP_2. Consequently, protein kinase B (PKB)/Akt, through its active site, binds to PIP_3, enabling PDK1 phosphorylation. This modification is crucial for activating mTORC1, promoting gene expression processes and cell proliferation.[34]

Wnt/β-Catenin Pathway

The Wnt pathway has two branches, known as canonical and non-canonical. The non-canonical one is independent of factors linked to β-catenin, while the canonical one involves β-catenin translocation to the nucleus to activate transcription factors.[35] The Wnt pathway controls cell proliferation, and the non-canonical branch uniquely regulates cell polarity and tumorigenic migration.[36] Wnt ligands in the extracellular environment activate the canonical branch. In their absence, an off-state prevails, directing β-catenin to proteasomal complexes and preventing cytoplasmic accumulation. In an on-state, Wnt ligands bind to Frizzled receptors and other ligands (such as proteins related to low-density lipoproteins 5 and 6 - LRP5/6), phosphorylating β-catenin proteasome complex groups and thus preventing its degradation.[37] In the non-canonical pathway, Wnt ligands connect to Frizzled receptors along with co-receptors of tyrosine kinase proteins, initiating a reaction cascade involving GTPases, Ras-like proteins (RhoA), and associated kinase pathways (ERK/JNK/MAPK),[38] with effector actions on cytoskeletal rearrangement, cell motility, and polarity.

Tumor Biomarkers

Biomarkers are molecules frequently associated with tumorigenesis and tumor progression, playing a crucial role in regulating malignancy through various molecular mechanisms. They encompass a wide range of compounds, including proteins, glycans, glycoproteins, genetic variants, enzymes, and long non-coding RNAs linked to cancer progression[39] (Table 4).

Table 4 Main Types and Specific Biomarkers in Tumors

Type of Biomarker	Tumor	Biomarker
Predictive	Breast	BRCA1/2 HER2 TOP2A
	Colorectal	RAS BRAF
	Squamous cell carcinoma	HER KRAS
	Lung	ROS1 BRAF V600E
	Melanoma	BRAF V600E/K
Prognosis	Breast	HER2 TOP2A 70-gene expression profile 58-gene RNA expression profile
	Prostate	tPSA
	Myeloid leukemia	EGR1
Diagnosis	Bladder	Chromosome aneuploidy of 3, 7, 17 Loss of 9p21
	Breast	MG CK19
	Colorectal	Hemoglobin assay
	B-cell lymphocytic leukemia	Alpha satellite region 12p11.1-q11
	Ovarian	BRCA1/2 Mutation
	Prostate	PCA3

At the forefront of biomarker research are long non-coding RNAs, such as *MALAT1* and *HOTAIR*, which have been implicated in various cancer types due to their ability to regulate gene expression and influence processes like cellular proliferation and migration. Additionally, the enzyme butyrylcholinesterase (BuChE) is notable in this context. Low levels of BuChE have been associated with poorer prognoses in colorectal cancer cases, suggesting that the dysregulation of its activity may adversely affect disease progression.[40]

While some biomarkers exhibit similarities across different tumor types, many are tissue-specific, making the identification of these biomarkers critical for clinical research. The association of biomarkers with unfavorable prognoses stimulates the development of targeted therapies aimed at improving patient survival and clinical outcomes.

Angiogenesis and Tumor Metastasis

Angiogenesis is a process in which tumor vascular networks emerge and integrate with the body's vascular system, providing macro and micronutrients necessary for tumor progression. This process begins with the association of endothelial cells with the tumor, followed by the development of a complex capillary network, which integrates with the normal capillary vessels, being essential for tumor growth and subsequent metastasis.[41] Typically, quiescent endothelial cells are activated by elevated levels of pro-angiogenic factors such as VEGF. Following activation by VEGF, other critical components are produced, including factors related to platelet emergence and VEGF receptors (VEGFR). The process proceeds with Notch pathway inhibition in the vascular system, stabilizing and modulating natural angiogenesis. The destabilization and loss of control of the angiogenesis process reshape the vascular system in the tumor region, giving rise to new vessels associated with the tumor.[42]

Angiogenesis is the primary mechanism in the metastatic process, being associated with tumors classified as malignant. Metastasis is defined as a process in which a primary tumor, following immediate integration with local lymph nodes, spreads to extra local spaces through cell evasion and inhibition of antitumorigenic factors.[43]

Metastasis initiates when single cells or cell clusters invade nearby lymph nodes or disseminate to distant tissues and organs via tumor-associated vessels.[44] Tumor cells rapidly adapt to new environments due to their genomic instability. This adaptation involves physical adaptations, such as cytoskeletal and plasma membrane remodeling, and gene expression of proteins involved in desmosomal processes and overall cell adhesion.[45] Hypoxia- and anoxia-resistant genes are also involved, such as Hypoxia-Inducible Factor (HIF)-induced signaling cascade and Hypoxia-Response Element (HRE) genes, which stimulate metabolism based on anaerobic glycolysis in tumor cells.[46] Recent investigations have also highlighted the role of tumor-associated macrophages that promote angiogenesis through lactate-secreting tumor cells, facilitating distant metastases in HER2+ molecular subtype breast tumor models.[47]

Tumor Microenvironment

Tumor cells and the environment surrounding the adjacent neoplastic tissue collaborate to tumor progression. This tumor microenvironment consists of various cell subpopulations, including stromal cells, immune cells, endothelial cells, fibroblasts, and adipocytes, along with hundreds of associated molecules that paradoxically inhibit and induce tumor malignancy.[48]

Endothelial Cells

Tumor-associated endothelial cells develop mechanisms from in situ to invasive stages, including cytokine secretion, receptor activation on tumor cells, and reducing cytotoxic T cell responses.[49] They also modulate tumor metabolism via paracrine factor interactions. Single-cell RNA-Seq studies by Tirosh et al revealed a link between these cells and Notch signaling through carbonic anhydrase 4 (CA4), highlighting their role, alongside fibroblasts, in regulating angiogenesis within the tumor microenvironment.[50–52]

Fibroblasts

Fibroblasts are crucial in homeostasis, involved in mechanical responses, physiological changes, and repair processes.[53] In the tumor microenvironment, they promote cancer progression.[54] Cancer-associated fibroblasts (CAFs) regulate tumor progression and are key drivers of poor prognosis in many cancers, being linked to therapeutic resistance and disease recurrence.[55]

Fibroblast-derived peptide factors suppress immune cells, promoting differentiation, proliferation, and neoplastic growth.[56] They stimulate pro-inflammatory cytokines like IFN-γ, TNF-α, and IL-1, which suppress T-lymphocyte function.[57]

Adipocytes

Adipocytes primarily influence tumor development in breast and abdominal cavity organ tissues, including the gastrointestinal tract. They contribute to tumor cell proliferation[58] by supplying metabolic resources, such as long-chain fatty acids, which are catabolized via β-oxidation to increase ATP production, thus enhancing tumor cell proliferation.[59]

Obesity, influenced by both environmental and genetic factors, is a significant risk factor for developing endometrial and breast cancers,[60] due to adipocyte hypertrophy and hormonal imbalances in these tissues. Cytokine and growth factor-mediated signaling pathways in adipocytes, including Interleukin-6 (IL-6), Tumor Growth Factor Beta (TGF-β), and VEGF, along with increased leptin and decreased adiponectin levels, trigger tumorigenesis and tumor progression.[61,62]

Immune System Cells

Innate and adaptive immune cells play dual roles in the tumor microenvironment as tumor-antagonizing (TaICs) or tumor-promoting (TpICs) cells.[63] TaICs, including CD8+ and CD4+ T cells, NK cells, macrophages, and neutrophils, combat tumors through cytokine release, apoptosis induction, and the production of pro-inflammatory cytokines.[63–65] Conversely, TpICs, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), inhibit cytotoxic T cells and promote angiogenesis by producing ECM-degrading metalloproteinases and VEGF.[63–65]

The complexity of immune system cells and their tumor-related actions remains poorly understood. Tumor progression is facilitated by evasion mechanisms like the PD-1/PDL-1 axis, where tumor cells express PDL-1 to inhibit cytotoxic T cell activity by binding to T cell PD-1 receptors.[66–69] Additionally, antagonist cells may exhibit paradoxical roles in tumor progression, such as T-cell exhaustion from persistent antigen exposure,[67,70] limited NK-cell activity due to immune checkpoint inhibitors,[71] and the controversial role of B cells, which can influence tumor progression by inducing angiogenesis and inhibiting cytotoxic T cells.[72]

Emerging Tumor Treatments

Cancer, a complex and heterogeneous group of diseases, has historically posed significant challenges for medical treatment. While tumor resection dates back to antiquity, modern cancer treatment emerged in the 20th century with advancements in surgical techniques and the introduction of radiotherapy and chemotherapy.[73] Some discoveries such as the use of mustard gas in tumor cells, identified through cytological analyses showing leukopenia in soldiers during the World Wars, were quite unexpected.[74]

The surge in technological innovation in the late 20th century significantly altered classic tumor treatments. For instance, while conventional radiotherapy is still widely used, four-dimensional image techniques tracking tumor movement and position are now employed to significantly reduce damage to adjacent healthy cells also affected by the treatment.[75] Currently, advanced therapies, including immunotherapy and *Chimeric Antigen Receptor T Cell* therapy (CAR-T cell therapy), offer personalized treatment options for various cancer types.

Over the past three decades of biomedical research, more than 100 antibodies have been approved for cancer immunotherapy. These antibodies exert their therapeutic effects through various mechanisms, including: (*i*) signal pathway inhibition (SPI), (*ii*) antibody-dependent cell-mediated cytotoxicity (ADCC), (*iii*) complement-dependent cytotoxicity (CDC), and (*iv*) antibody-dependent cellular phagocytosis (ADCP).[76]

The main SPI antibodies include cetuximab and panitumumab, which act through competitive inhibition of the Epidermal Growth Factor Receptor (EGFR) and antagonistic action, inducing EGFR internalization, respectively.[77] Among ADCCs, loncastuximab targets the CD19 antigen primarily expressed on B cells, making it an effective immunotherapy for lymphomas.[77]

In the past decade, immunotherapy has focused on two main pathways of tumor evasion: the PD-1/PDL-1 axis (with Pembrolizumab and Atezolizumab, respectively) and the CTLA-4 immune *checkpoint* pathway Ipilimumab), the latter being characterized for inhibiting the cytotoxic actions of T lymphocytes by interacting with their ligands (CD80 and CD86) present on antigen-presenting cells.[77]

CAR-T Cells in Cancer Treatment

CAR-T Cell Therapy has emerged as a paradigm-shifting revolution in Oncology, offering a novel approach to treating refractory tumors. By genetically reprogramming a patient's T cells, this therapy provides a targeted immune response

against malignant cells, radically changing the therapeutic landscape. Its technological foundation involves producing chimeric antigens linked to T cells, typically through transgenic insertion assays.[78] These synthetic receptors must conform to a rigid structural template, consisting of four main components: an extracellular antigen-binding domain, a hinge region, a transmembrane domain, and intracellular signaling domains.[79,80]

The cornerstone of CAR-T cell therapy lies in the specific interaction of chimeric antigens with proteins predominantly or exclusively expressed in tumors, rendering this therapy one of the most prominent in recent years.

In a constantly developing field, to this date, the Food and Drug Administration (FDA) has approved six CAR-T cell treatments in the USA,[81] namely:

- Kymriah™ (tisagenlecleucel): for patients with acute lymphoblastic leukemia, B-cell lymphomas, and follicular lymphomas, featuring a chimeric antigen receptor for CD19;
- Yescarta™ (axicabtagene ciloleucel): for patients with B-cell lymphomas and follicular lymphomas, featuring a chimeric antigen receptor for CD19;
- Tecartus™ (brexucabtagene autoleucal): for patients with mantle-cell lymphoma and acute lymphoblastic leukemia, featuring a chimeric antigen receptor for CD19;
- Breyanzi™ (lisocabtagene maraleucel): for patients with B-cell lymphomas, featuring a chimeric antigen receptor for CD19;
- Abecma™ (idecabtagene vicleucel): for patients with multiple myeloma, featuring a chimeric antigen receptor for BCMA;
- Carvykti™ (citacabtagene autoleucel): for patients with multiple myeloma, featuring a chimeric antigen receptor for BCMA.

Extracellular Vesicles and Tumors

Extracellular Vesicles (EVs), involved in autocrine, paracrine, and endocrine signaling processes, are crucial for embryonic developmental stages, influencing cell differentiation and proliferation via Wnt and Hedgehog protein gradients,[82] neural crosstalk,[83] and immunological signaling, activating Antigen Presenting Cells (APCs) and T lymphocytes.[84] Given their association with cell proliferation, migration, differentiation, and immune cell activation, EVs have been closely linked to tumorigenesis and tumor progression. Moreover, the discovery of EVs in liquid tumor biopsies has solidified their role as critical biomarkers. Substantial differences in surface proteins and intracellular molecules such as miRNAs between early and advanced-stage tumors render EVs an invaluable tool for cancer prognosis.[85]

Tumor cells release significantly more EVs than normal cells, highlighting their crucial role in tumor-microenvironment communication and subsequent malignant progression.[86] This EV-mediated communication is involved in angiogenesis induction, release of pro-metastatic factors and immune suppression, notably through actions of siRNAs,[87] induction of chemokine secretion by macrophages and synthesis of TGFβ,[88] in addition to EMT through EVs released by tumor cells under hypoxic conditions.[89]

Additionally, EVs actively contribute to ECM remodeling within the tumor microenvironment. By transferring proteins such as fibronectin, annexins, and integrins to the tumor stroma via exosomes, EVs facilitate tumor invasion through the formation of new ECM scaffolds.[90,91]

Over the past two decades, EV research has significantly advanced, leading to their dual application in cancer therapy. EVs serve not only as therapeutic targets but also as versatile drug delivery vehicles capable of directly targeting tumor cells and the microenvironment.[41] Their small size, versatile cell-entry mechanisms, biocompatibility and potential signal peptide modification in their structures render EVs ideal candidates for drug delivery systems.[92]

Pan-Cancer Research

Despite the numerous cancer divergencies and its establishment as a heterogeneous set of pathologies, the landscape emerging from a significant number of convergences is as rich and complex as its individualized studies.

Over the past two decades, extensive research has shaped the field of pan-cancer analysis; however, the publication of "Pan-cancer analysis of whole genomes" initiative, published in Nature was indeed a pivotal moment for the field.[93] This publication

sparked increased research in the field, coinciding with similar efforts by Cell Press through TCGA Consortium, funded by the National Cancer Institute and the National Human Genome Research Institute. These efforts aim to comprehensively analyze cancer by examining tumor cells, their molecular similarities across different tumor types, associated oncological processes involved signaling pathways, and the genetic and epigenetic changes driving human tumor development.

Tumor Cell-of-Origin Patterns

Cell-of-origin refers to the genetic changes a normal cell undergoes as it becomes cancerous. Understanding these alterations is crucial for pan-cancer analysis, identifying clusters with associated modifications across tumors with respect to aneuploidies, DNA hypermethylation, transcriptional expression of coding and non-coding genes, microRNA transcription, and protein expression.[94] Hoadley et al, in their analysis of 10,000 tumor samples, highlighted a characteristic heterogeneity regarding aneuploidy, dividing 33 types of tumors into 10 different clusters, particularly noting those with a higher aneuploidy index. Regarding epigenomic changes, over 3000 tumor samples exhibited hypermethylation in CpG islands, forming 25 clusters with similarities in methylation positions; therefore, the epigenomic mechanism is directly associated with similar cellular populations, governed by pre-existing transcription programs.

In turn, mRNA analyses revealed insights into gene expression linked to tumorigenesis. Similar morphology cells showed similar transcriptional potential compared to cells of more distinct morphologies and origins. The results for microRNAs were more heterogeneous, indicating a more effective integration among more distantly related tumors.[94]

By linking oncogenic processes, researchers can more precisely identify specific changes across different tumor types and search for antitumoral drugs for oncological therapy. In 2018, using TCGA data, Sanchez-Vega et al conducted a meticulous analysis that established mechanistic relationships between ten classic pathways studied in oncology.[95] Their findings included:

- A high average RTK-RAS signaling pathway alterations across all analyzed tumor types, totaling 46% of the studied samples;
- KRAS is the most frequently altered gene (9% of the samples);
- BRAF gene alterations were found in over 50% of the melanoma and thyroid carcinoma samples;
- Synergistic relationships between different pathways in different tumor types directly influence each other's changes.

Thorsson et al shed light on the immune system's role in tumor initiation. By extensively analyzing TCGA data across 33 cancer types, Thorsson et al identified six distinct immune response patterns within the neoplastic environment, namely: wound healing, IFN-γ dominant, inflammation, lymphocyte depletion, immune silencing and TGF-β dominant. These classifications were based on analyzing markers of innate and adaptive immune systems and exploring the immune population heterogeneity in different tumor types, the presence of aneuploidies and gene expression and co-expression of modulatory genes.

The data suggest a strong link between immune responses and patient survival across different tumor types. Additionally, the study revealed how immune evasion varies across different tumor-associated microenvironments. Intriguingly, tumors with dominant immune responses displayed a wider variety of B and T cell lymphocyte receptors (TCRs and BCRs), leading to a more effective response against the tumor environment. In contrast, other tumor types favored immune escape and tissue inflammation.[96]

Based on data from the Pan-Cancer Analysis of Whole Genomes initiative, the results have increasingly correlated somatic mutations as significant factors in tumor initiation. The analyses revealed over 43 million somatic alterations, 2.4 million somatic indels, and 19.4 million retrotransposition events across the data.[93]

Among the findings, key points of interest in biomedical and clinical research included:

- Dual inactivation of TP53 alleles triggers instability in tumor suppressor genes;
- Three processes are associated with genomic reconfiguration in tumor emergence: chromoplexy, kataegis, and chromothripsis.

Chromoplexy, a process of inappropriate DNA reorganization through repair mechanisms among different chromosomes, was identified in over 17% of the samples, especially in prostatic adenocarcinomas and lymphoid tumors. *Kataegis*,

characterized by defined sites of gene hypermutations, was found in over 60% of tumors, notably in lung carcinomas, bladder tumors, melanomas, and sarcomas. Chromothripsis, defined by extensive fragmentation of a chromosome into small fragments, followed by random reorganization and reconnection, occurred in 22% of the total samples, with a higher association with sarcomas, glioblastoma, and breast adenocarcinoma.[93]

Pan-cancer research also extensively studies the tumor microenvironment. Its heterogeneity poses a significant challenge, addressed through the use of single-cell RNA-Seq, in a remarkable effort by Nofech-Mozes et al. By analyzing over 300,000 tumor microenvironment-related cells across 19 common tumor types, using comparative machine learning, researchers extracted gene expression data from tumor cells and surrounding tissues. This allowed them to create cell/tumor type maps identifying the tissue origin of tumors and understanding how cells interact within tumors.[97]

In short, pan-cancer research is essential due to the complex and varied nature of tumors, with numerous regulatory and modulatory mechanisms. Therefore, by studying multiple cancer types together, researchers can identify commonalities and differences, leading to improved diagnosis, prognosis, and treatment. This approach holds the promise of more accurate and personalized therapies, which is crucial for a medium- to long-term reduction in global mortality rates due to different cancers.

Results and Discussion

We undertook a global analysis of epidemiological, genetic, and protein data across 19 cancer types using three large public repositories, namely: the Pan-cancer analysis of Whole Genomes (ICGC/TCGA), China Pan-cancer, and Metastatic Solid Cancers. These datasets combined include over 13,616 tumor samples, with 21% representing advanced (stage IV), or metastatic tumors (Figure 2A and B).

A standardized patient profile was established among the samples, gathering information on cell type, recurrence site and whether the tumor was primary or metastatic. By analyzing patient records, which could include multiple samples per patient, the dataset contained more samples and patients with primary tumors, followed by metastatic tumors (Figure 2C and D).

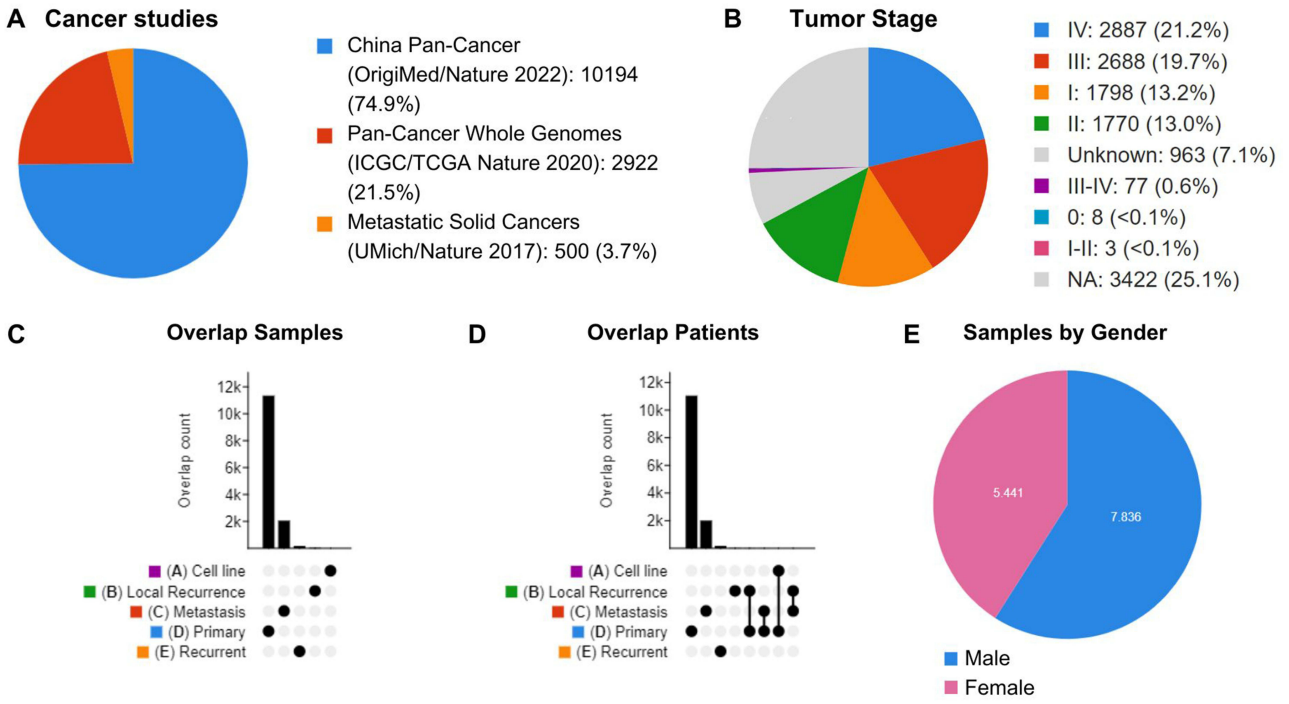

Figure 2 Datasets of Pan-cancer. (**A**) Pan-cancer repositories used for in silico analyses; (**B**) Samples divided by tumor stage; (**C** and **D**) Correlations of sample and patient numbers and primary tumor site, metastasis, recurrence and site of recurrence; (**E**) Samples divided by gender.

By overlapping patients, a correlation is observed between tumor recurrence and the original and subsequent tumor locations. Recurrences often appeared at the initial treatment site (eg, breast cancer returning in the breast) or in tissues similar to the original tumor (eg, breast cancer recurring in lymph node-rich areas or reproductive organs, such as salivary gland and ovarian tumors).

Regardless of age and ethnicity, gender analysis revealed that women are more likely to develop cancer than men (six out of 10), excluding non-melanoma skin cancer. Specifically, breast cancer is the most common in women, while lung cancer (excluding melanomas) is the most prevalent in men. These findings also align with smoking rates, wherein 37% of smokers worldwide are men, and only 7% are women, highlighting a correlation with lung tumors.[98]

Results on ethnic epidemiology and mutated gene frequency provide insights into the convergence of tumors worldwide. The top 10 genes with the highest alteration frequency across all 20 tumor types occur in all global populations. However, the BICRA gene, involved in chromatin remodeling and proposed as a glioma suppressor gene, shows a higher mutation frequency in South Asian populations. This observation requires more in-depth longitudinal clinical analyses to establish a relationship between the incidence of this tumor type in that population.

An analysis of the main types of genetic alterations across the 20 tumor types studied here reveals intriguing patterns in tumor development and progression. The association of ADAM family genes with ECM reorganization is particularly enriched in pancreatic tumors. Such a correlation links the progression of this tumor type to cell adhesion dysregulation and potential aggressiveness, primarily through metastasis, as well as a morphological remodeling of tumor cells, potentially through a more effective EMT mechanism.

Protein interaction predictions were conducted among the 48 most mutated genes in the tumor types studied here (Table 5). Within the analyzed sample size (>13,000), 40% of these genes are not categorized as tumor-associated genes, highlighting the importance of bioassays to correlate them with tumorigenesis and tumor progression.

Protein interaction pathways were clustered using the k-means algorithm, an unsupervised learning method, to group proteins based on family similarity and predicted interactions from in silico analyses (Table 6 and Figure 3A–E). Based on these predictions and established pathways among proteins within the same clusters or through protein interactions across different clusters, the following hypotheses can be postulated:

Table 5 Frequency of the Main Mutated Genes in Tumors in 13777 Samples

Gene	Mutations	Number of Samples	Frequency	Is a Classically Cancer Gene?
TP53	7450	6992	52.30%	Yes
LRP1B	2347	1675	12.50%	Yes
KRAS	2046	2023	15.10%	Yes
APC	2036	1410	10.50%	Yes
KMT2D	1499	1168	8.70%	Yes
EGFR	1408	1191	8.90%	Yes
PIK3CA	1375	1209	9.00%	Yes
ARID1A	1354	1157	8.60%	Yes
FAT4	1341	964	7.20%	Yes

(*Continued*)

Table 5 (Continued).

Gene	Mutations	Number of Samples	Frequency	Is a Classically Cancer Gene?
KMT2C	1150	876	6.50%	Yes
TERT	993	966	7.20%	Yes
FAT1	894	710	5.30%	Yes
SMAD4	857	798	6.00%	Yes
RB1	830	773	5.80%	Yes
CDKN2A	795	763	5.70%	Yes
ATM	792	636	4.80%	Yes
PRKDC	771	622	4.60%	Yes
CTNNB1	752	694	5.20%	Yes
NF1	744	597	4.50%	Yes
NOTCH1	717	618	4.60%	Yes
PTEN	711	588	4.40%	Yes
PREX2	683	575	4.30%	Yes
PBRM1	643	552	4.10%	Yes
CREBBP	637	551	4.10%	Yes
ARID2	627	527	3.90%	Yes
SMARCA4	605	539	4.00%	Yes
FBXW7	590	522	3.90%	Yes
BRCA2	578	452	3.40%	Yes
SETD2	575	477	3.60%	Yes
NOTCH3	558	480	3.60%	Yes
ERBB4	547	485	3.60%	Yes
ATRX	543	457	3.40%	Yes
ROS1	527	415	3.10%	Yes
EPHA5	511	447	3.30%	Yes
SLIT2	508	440	3.30%	Yes
SPEN	508	415	3.10%	Yes
GRIN2A	501	431	3.20%	Yes
KMT2A	493	413	3.10%	Yes
GLI2	482	431	3.20%	No
SPTA1	1297	1065	8.00%	No
LRP2	1212	937	7.00%	No

(*Continued*)

Table 5 (Continued).

Gene	Mutations	Number of Samples	Frequency	Is a Classically Cancer Gene?
MUC16	874	356	13.30%	No
FAT3	1473	1123	8.40%	No
LRP1	759	612	4.60%	No
FAM135B	752	642	4.80%	No
GLI3	606	525	3.90%	No
TTN	1698	726	22.80%	No

Table 6 Protein Clusters and Main Associated Mechanisms

Subclusters	Associated Proteins	Significant Associated Mechanisms
1	PIK3CA; ARID1A; SMAD4; CDKN2A; CTNNB1; FAT1; KRAS; APC; TP53; RB1; TERT; EGFR; APOB; LRP2	**GO Biological Process** – Regulation of G1/S Transition of Mitotic Cell Cycle; **GO Molecular Function** – Ubiquitin Protein Ligase Binding; GO Cellular Component – Clathrin-Coated Endocytic Vesicle Membrane PPI enrichment p-value: $7{,}16e^{-10}$
2	FSIP2; CSMD1; CSMD3; XIRP2; SPTA1; RYR2; OBSCN; KMT2; TTN; RYR1; RYR3; DNAH3; FLG	**GO Biological Process** – Histone H3-K4 Methylation **GO Molecular Function** – Histone H3 Methyltransferase Activity PPI enrichment p-value: $1e^{-16}$
3	ADGRV1; PCDH15; EYS; USH2A; DNAH11; PKHD1L1	**GO Molecular Function** – Myosin Binding PPI enrichment p-value: $4e^{-15}$
4	ABCA13; FAT3; FAT4; ZFHX4; MUC16; PCLO; MUC19; MUC17; HMCN1; DNAH5; LRP1B; KMT2D	**GO Biological Process** – Heterophilic Cell-Cell Adhesion MUC16 alterations (CA-125 associated oncotic antigen) **Reactome** – defects in GALNT12 (Akt signaling pathway alterations) PPI enrichment p-value: $1e^{-16}$

- CTNNB1 possibly modulates the cell cycle by alterations in the Wnt/Beta-Catenin signaling pathway via APC and activating the MAPK pathway and others involved with proteins encoded by the KRAS and RB1 genes, thus relating to uncontrolled proliferation processes. It also directly acts on negative feedback mechanisms in apoptotic processes, in addition to the positive feedback of cell migration processes via the expression of atypical cadherins (FAT1);
- KMT family proteins (such as KMT2A, KMT2B, KMT2C, KMT2D and KMT2E) are mainly responsible for tumorigenesis processes via epigenetic modulation by methylation of histones at lysine residues;

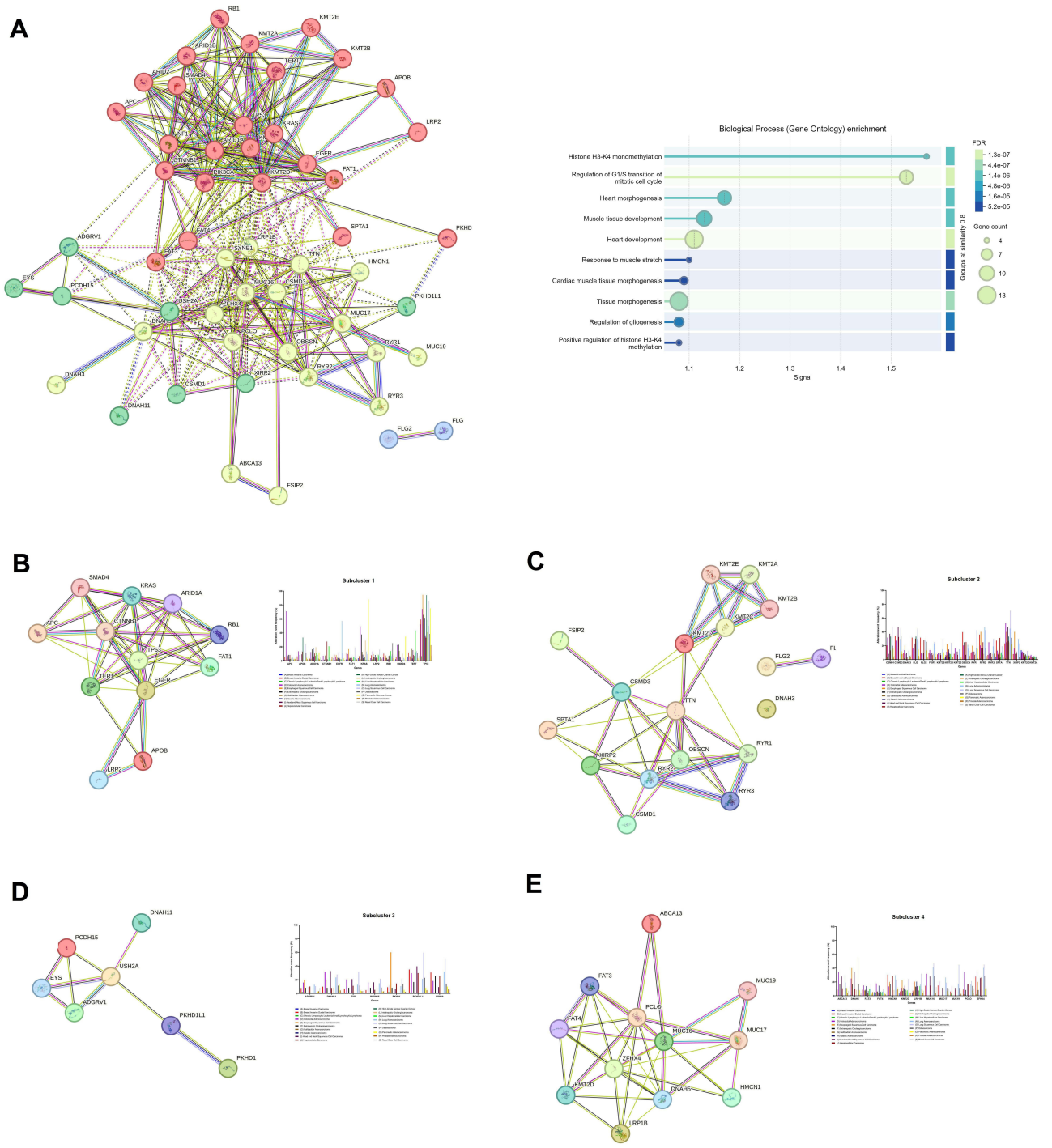

Figure 3 Protein predictions networks by StringDB Analysis. Protein prediction analyses via *StringDB*, delimiting the (**A**) global interactions between the main protein-coding genes mutated in the pan-cancer analyses and Ontological analysis of Biological Processes (main cluster) and (**B**–**E**) local interactions, through subclusters 1–4, respectively, with data on the mutational frequency of the genes of each subcluster in the 19 types of tumors analyzed. The protein interactions evidenced in the clusters indicate relationships evidenced both experimentally (Purple line) and predicted in silico (Red line - presence of fusion evidence; Green line - neighborhood evidence; Blue line - cooccurrence evidence; Yellow line - textmining evidence; Light blue line - database evidence; and Black line - coexpression evidence). The interactions between subclusters are seen through the dashed lines.

- RB1 is closely linked to the regulation of tumorigenic processes in glioblastomas through interactions with proliferation mechanisms involving alterations in genes such as TP53, KRAS, and EGFR;
- HMCN1 may be involved in increased tumor invasion through modulation of tumor-associated fibroblasts.

Gender-dependent enrichment analyses were also performed and showed that the main genes correlated with tumorigenesis have similar expression in men and women, but genes such as DNAH5, ADGRV1 (in women) and EYS (in men) appear mutated more frequently (Figure 4A–D).

Ultimately, tumor convergence, beyond the search for novel proteins or associated molecules, is rooted in well-known genes involved in tumoral processes. However, the study of these genes should not only focus on proliferation pathways and the various already well-understood processes but also on the relationships between different genes not directly related to tumors. Various genes have interactions in tumors that are distinct in terms of the main pathways associated with their malignancy, progression and others secondary processes, as shown in Figure 5:

The relationship between these processes and the genes involved and delimited by pan-cancer analyses demonstrates the intricate gene network involved in the processes of tumorigenesis and tumor progression, bringing light to new molecules that can help in the landscape of basic, applied and clinical oncology research.

These findings underscore the necessity of considering genes previously unrecognized in the tumor context yet exhibiting altered expression across various cancer types. While the heterogeneity of available data poses challenges, it also provides opportunities to uncover new molecular interactions and genetic networks pivotal to tumorigenesis. Integrative bioinformatics approaches can identify innovative therapeutic targets and prognostic biomarkers, enhancing our understanding of the underlying mechanisms of cancer. Therefore, a critical and thorough analysis of these data is essential to reveal hidden biological potential and facilitate significant advancements in oncology, ultimately improving diagnostics, prognostics, and personalized therapies for patients.

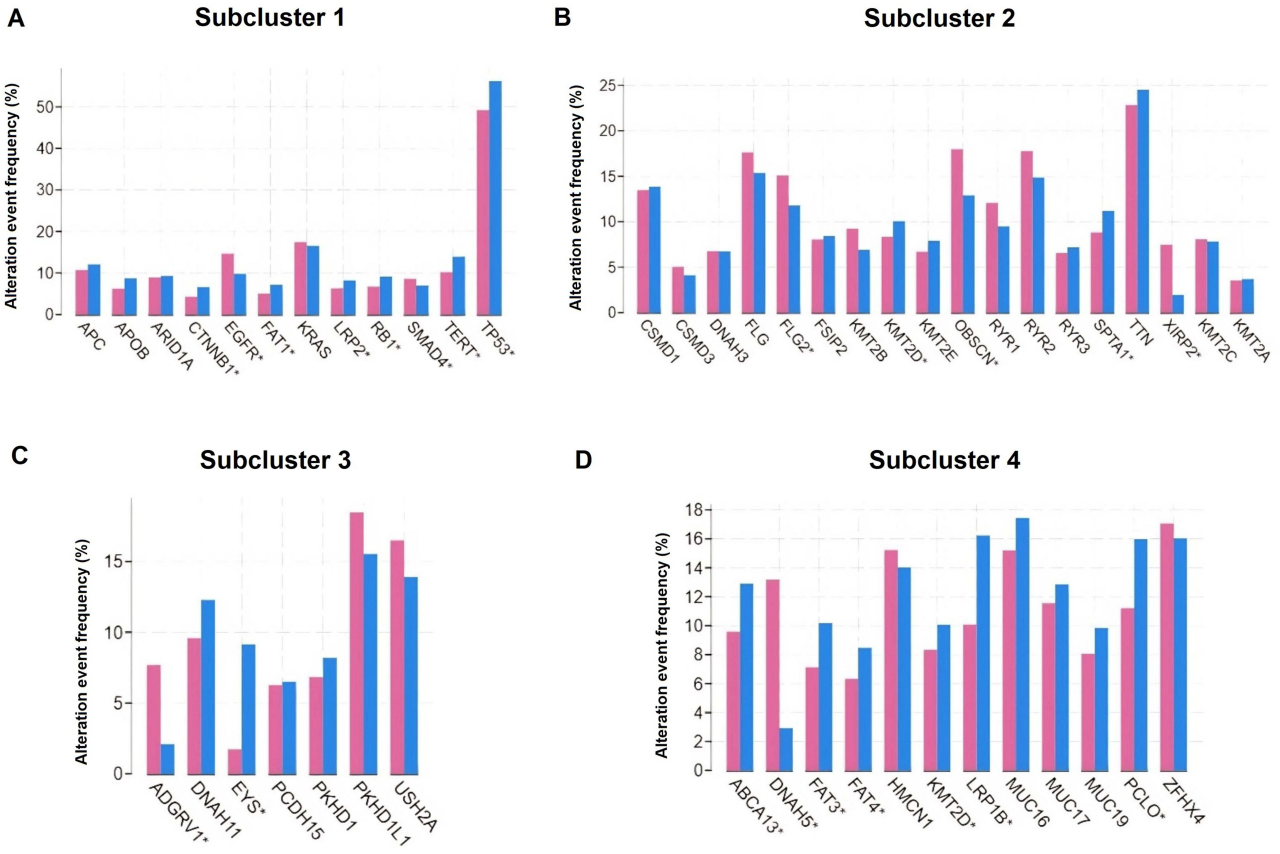

Figure 4 Frequency of gene mutations of the main genes of the protein interaction subclusters by gender. (**A**–**D**) Mutational frequency of genes in each subcluster of male (blue bars) and female (pink bars) genders, according to the analyzed subclusters 1–4.

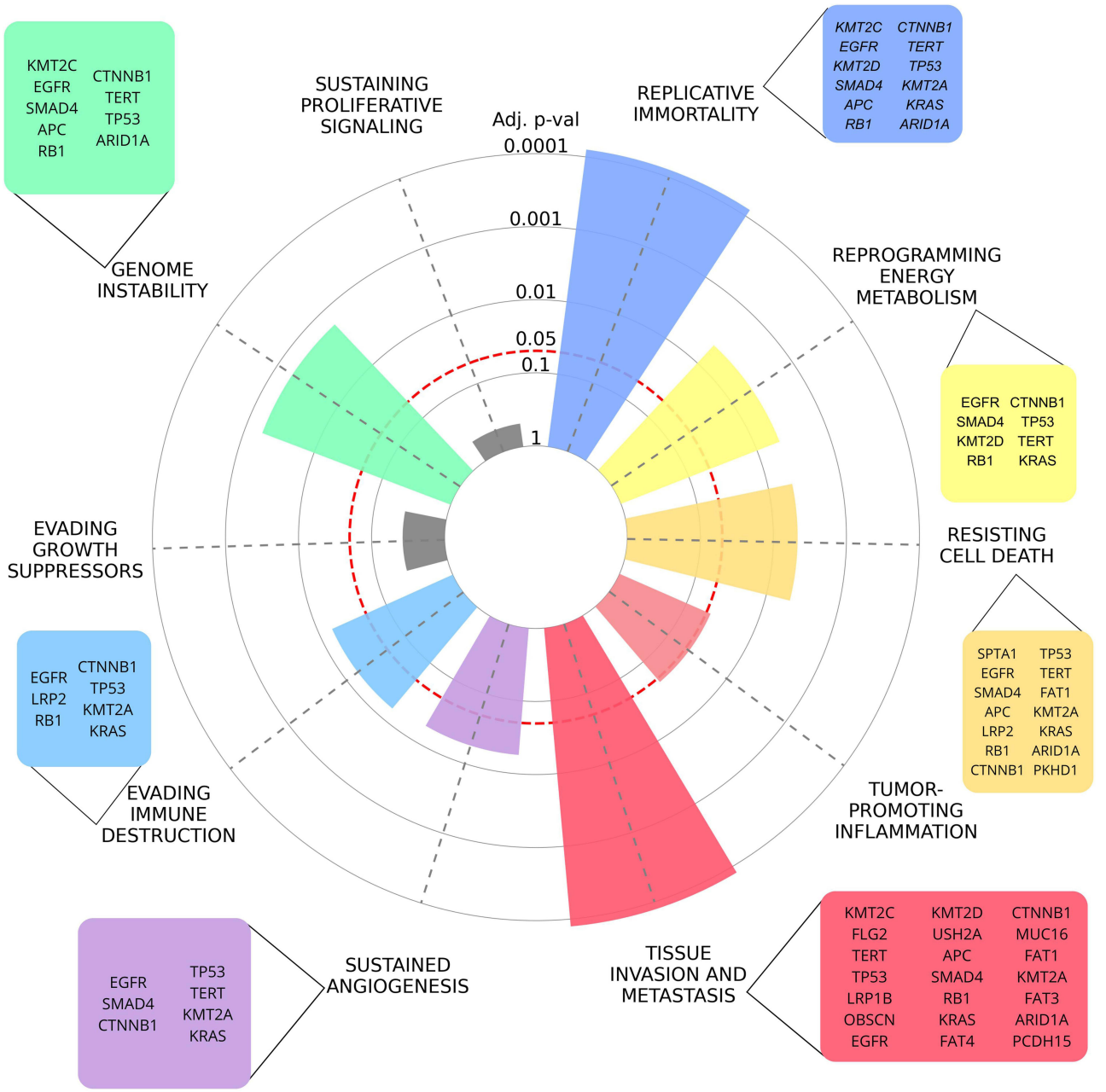

Figure 5 Tumor hallmarks associated with the analyzed genes. Association between the main tumor hallmarks and the most frequently mutated genes in the pan-cancer analyses performed.

Conclusions

Our literature review reveals a complex network of relationships among different tumor types, particularly in terms of molecular interactions and pathways activation, triggering uncontrolled proliferation, migration, and invasion mechanisms. Studies have mainly focused on the convergences that different molecular types and subtypes share within the context of pan-cancer.

Convergence patterns have also been identified and highlighted in seminal works, with significant additions from multi-omics data analyses, demonstrating the existence of diverse regulatory networks and protein interactions with potential clinical interest in the future.

Comparative analyses of different tumor types provided a comprehensive overview of molecular similarities and disparities, enabling the identification of potential therapeutic targets applicable across multiple oncological scenarios, such as those

involving CTNNB1, APC, KMT2D and RB1 genes. This integrated approach underscores the importance of translational research and precision medicine for a better understanding of the shared and distinct molecular basis across various cancer types.

In conclusion, our findings underscore the intricate interplay between epidemiological, genetic and molecular factors driving tumorigenesis, offering a holistic view of pan-cancer oncology. This profound knowledge is essential for designing more targeted and effective therapeutic interventions, ultimately leading to improved control and treatment of the diverse spectrum of cancers.

Funding

This work was supported by grants from the following Brazilian research funding agencies: CAPES (Grant No. 88887.646286/2021-00), FAPESP (Thematic Project No. 2016/05311-2) and CNPq (Grant No. 457601/2013-2, 401430/2013-8 and INCT-Regenera).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Cancer (IARC) TIA for R on. Global Cancer Observatory. Available from: https://gco.iarc.fr/. Accessed August 9, 2024.
2. Lin J, Ma L, Zhang D, et al. Tumour biomarkers—tracing the molecular function and clinical implication. *Cell Proliferation*. 2019;52(3):e12589. doi:10.1111/cpr.12589
3. Anderson NM, Simon MC. The tumor microenvironment. *Curr Biol*. 2020;30(16):R921–R925. doi:10.1016/j.cub.2020.06.081
4. Hsu PP, Sabatini DM. Cancer cell metabolism: Warburg and beyond. *Cell*. 2008;134(5):703–707. doi:10.1016/j.cell.2008.08.021
5. Henke E, Nandigama R, Ergün S. Extracellular matrix in the tumor microenvironment and its impact on cancer therapy. *Front Mol Biosci*. 2020;6. doi:10.3389/fmolb.2019.00160
6. Sobierajska K, Ciszewski WM, Sacewicz-Hofman I, Niewiarowska J. Endothelial cells in the tumor microenvironment. In: Birbrair A, editor. *Tumor Microenvironment: Non-Hematopoietic Cells*. Springer International Publishing; 2020:71–86. doi:10.1007/978-3-030-37184-5_6
7. Killock D. Sequencing cells of the immune TME. *Nat Rev Clin Oncol*. 2018;15(9):531. doi:10.1038/s41571-018-0069-0
8. Alexandrov LB, Kim J, Haradhvala NJ, et al. The repertoire of mutational signatures in human cancer. *Nature*. 2020;578(7793):94–101. doi:10.1038/s41586-020-1943-3
9. Li Y, Dou Y, Leprevost FDV, et al. Proteogenomic data and resources for pan-cancer analysis. *Cancer Cell*. 2023;41(8):1397–1406. doi:10.1016/j.ccell.2023.06.009
10. Hong SN. Genetic and epigenetic alterations of colorectal cancer. *Intestinal Res*. 2018;16(3):327–337. doi:10.5217/ir.2018.16.3.327
11. Puneet, Kazmi HR, Kumari S, Tiwari S, Khanna A, Narayan G. Epigenetic mechanisms and events in gastric cancer-emerging novel biomarkers. *Pathol Oncol Res*. 2018;24(4):757–770. doi:10.1007/s12253-018-0410-z
12. Amorim MO, Vieira MM, Gonçalves IV, Rhana P, Rodrigues ALP. Câncer de mama: reprogramação do metabolismo tumoral. *Rev Med Minas Gerais*. 2018;28(1):1–9. doi:10.5935/2238-3182.20180078
13. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646–674. doi:10.1016/j.cell.2011.02.013
14. Böttcher M, Baur R, Stoll A, Mackensen A, Mougiakakos D. Linking immunoevasion and metabolic reprogramming in B-cell–derived lymphomas. *Front Oncol*. 2020;10. doi:10.3389/fonc.2020.594782
15. Johnson DG, Walker CL. Cyclins and cell cycle checkpoints. *Annu Rev Pharmacol Toxicol*. 1999;39:295–312. doi:10.1146/annurev.pharmtox.39.1.295
16. Vermeulen K, Van Bockstaele DR, Berneman ZN. The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. *Cell Proliferation*. 2003;36:131–149. doi:10.1046/j.1365-2184.2003.00266.x
17. Ren B, Cam H, Takahashi Y, et al. E2F integrates cell cycle progression with DNA repair, replication, and G2/M checkpoints. *Genes Dev*. 2002;16(2):245–256.
18. Ajani JA, Song S, Hochster HS, Steinberg IB. Cancer stem cells: the promise and the potential. *Semin Oncol*. 2015;42:S3–S17.
19. Wu CI, Wang HY, Ling S, Lu X. The ecology and evolution of cancer: the ultra-microevolutionary process. *Annu Rev Genet*. 2016;50(1):347–369. doi:10.1146/annurev-genet-112414-054842
20. Dongre A, Weinberg RA. New insights into the mechanisms of epithelial–mesenchymal transition and implications for cancer. *Nat Rev Mol Cell Biol*. 2019;20(2):69–84. doi:10.1038/s41580-018-0080-4
21. Loh CY, Chai JY, Tang TF, et al. The E-cadherin and N-cadherin switch in epithelial-to-mesenchymal transition: signaling, therapeutic implications, and challenges. *Cells*. 2019;8(10):1118. doi:10.3390/cells8101118
22. Martin TA, Goyal A, Watkins G, Jiang WG. Expression of the transcription factors snail, slug, and twist and their clinical significance in human breast cancer. *Ann Surg Oncol*. 2005;12(6):488–496. doi:10.1245/ASO.2005.04.010
23. Onder TT, Gupta PB, Mani SA, Yang J, Lander ES, Weinberg RA. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. *Cancer Res*. 2008;68(10):3645–3654. doi:10.1158/0008-5472.CAN-07-2938
24. Serrano-Gomez SJ, Maziveyi M, Alahari SK. Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications. *Mol Cancer*. 2016;15(1):18. doi:10.1186/s12943-016-0502-x
25. Jolly MK. Implications of the hybrid epithelial/mesenchymal phenotype in metastasis. *Front Oncol*. 2015;5. doi:10.3389/fonc.2015.00155
26. Guo R, Luo J, Chang J, Rekhtman N, Arcila M, Drilon A. MET-dependent solid tumours — molecular diagnosis and targeted therapy. *Nat Rev Clin Oncol*. 2020;17(9):569–587. doi:10.1038/s41571-020-0377-z

27. Keshet Y, Seger R. The MAP kinase signaling cascades: a system of hundreds of components regulates a diverse array of physiological functions. In: Seger R, editor. *MAP Kinase Signaling Protocols*. 2nd ed. Humana Press; 2010:3–38. doi:10.1007/978-1-60761-795-2_1
28. Eblen ST. Chapter four - extracellular-regulated kinases: signaling from Ras to ERK substrates to control biological outcomes. In: Tew KD, Fisher PB, editors. *Advances in Cancer Research*. Vol. 138. Academic Press; 2018:99–142. doi:10.1016/bs.acr.2018.02.004
29. Plotnikov A, Zehorai E, Procaccia S, Seger R. The MAPK cascades: signaling components, nuclear roles and mechanisms of nuclear translocation. *Biochim Biophys Acta Mol Cell Res*. 2011;1813(9):1619–1633. doi:10.1016/j.bbamcr.2010.12.012
30. Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov*. 2009;8(8):627–644. doi:10.1038/nrd2926
31. Salajegheh A. *Angiogenesis in Health, Disease and Malignancy*. Springer International Publishing; 2016. doi:10.1007/978-3-319-28140-7
32. Pérez-Tenorio G, Alkhori L, Olsson B, et al. PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. *Clin Cancer Res*. 2007;13(12):3577–3584. doi:10.1158/1078-0432.CCR-06-1609
33. Balakrishnan A, Chaillet JR. Role of the inositol polyphosphate-4-phosphatase type II *Inpp4b* in the generation of ovarian teratomas. *Dev Biol*. 2013;373(1):118–129. doi:10.1016/j.ydbio.2012.10.011
34. Dowling RJ, Topisirovic I, Alain T, et al. mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. *Science*. 2010;328 (5982):1172–1176. doi:10.1126/science.1187532
35. Liu J, Xiao Q, Xiao J, et al. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. *Signal Transduct Target Ther*. 2022;7(1):3.
36. Clevers H. Wnt/β-catenin signaling in development and disease. *Cell*. 2006;127(3):469–480. doi:10.1016/j.cell.2006.10.018
37. Koni M, Pinnarò V, Brizzi MF. The Wnt signalling pathway: a tailored target in cancer. *Int J Mol Sci*. 2020;21(20):7697. doi:10.3390/ijms21207697
38. Gajos-Michniewicz A, Czyz M. WNT signaling in melanoma. *Int J Mol Sci*. 2020;21(14):4852. doi:10.3390/ijms21144852
39. Verdaguer H, Saurí T, Macarulla T. Predictive and prognostic biomarkers in personalized gastrointestinal cancer treatment. *J Gastrointest Oncol*. 2017;8(3):405–417. doi:10.21037/jgo.2016.11.15
40. Verras GI, Mulita F. Butyrylcholinesterase levels correlate with surgical site infection risk and severity after colorectal surgery: a prospective single-center study. *Front Surg*. 2024;11:1379410. doi:10.3389/fsurg.2024.1379410
41. Nowak-Sliwinska P, Alitalo K, Allen E, et al. Consensus guidelines for the use and interpretation of angiogenesis assays. *Angiogenesis*. 2018;21 (3):425–532. doi:10.1007/s10456-018-9613-x
42. Liu ZJ, Shirakawa T, Li Y, et al. Regulation of Notch1 and Dll4 by vascular endothelial growth factor in arterial endothelial cells: implications for modulating arteriogenesis and angiogenesis. *Mol Cell Biol*. 2003;23(1):14–25. doi:10.1128/MCB.23.1.14-25.2003
43. Fischer KR, Durrans A, Lee S, et al. Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. *Nature*. 2015;527(7579):472–476. doi:10.1038/nature15748
44. Aceto N, Bardia A, Miyamoto DT, et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell*. 2014;158 (5):1110–1122. doi:10.1016/j.cell.2014.07.013
45. Cheung KJ, Gabrielson E, Werb Z, Ewald AJ. Collective invasion in breast cancer requires a conserved basal epithelial program. *Cell*. 2013;155 (7):1639–1651. doi:10.1016/j.cell.2013.11.029
46. Chaturvedi P, Gilkes DM, Wong CCL, et al. Hypoxia-inducible factor–dependent breast cancer–mesenchymal stem cell bidirectional signaling promotes metastasis. *J Clin Invest*. 2013;123(1):189–205. doi:10.1172/JCI64993
47. Linde N, Casanova-Acebes M, Sosa MS, et al. Macrophages orchestrate breast cancer early dissemination and metastasis. *Nat Commun*. 2018;9 (1):21. doi:10.1038/s41467-017-02481-5
48. Xiao Y, Yu D. Tumor microenvironment as a therapeutic target in cancer. *Pharmacol Ther*. 2021;221:107753. doi:10.1016/j.pharmthera.2020.107753
49. Yang D, Guo P, He T, Powell CA. Role of endothelial cells in tumor microenvironment. *Clin Transl Med*. 2021;11(6):e450. doi:10.1002/ctm2.450
50. Cao Z, Ding BS, Guo P, et al. Angiocrine factors deployed by tumor vascular niche induce B cell lymphoma invasiveness and chemoresistance. *Cancer Cell*. 2014;25(3):350–365. doi:10.1016/j.ccr.2014.02.005
51. Tirosh I, Izar B, Prakadan SM, et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science*. 2016;352 (6282):189–196. doi:10.1126/science.aad0501
52. Kim N, Kim HK, Lee K, et al. Single-cell RNA sequencing demonstrates the molecular and cellular reprogramming of metastatic lung adenocarcinoma. *Nat Commun*. 2020;11(1):2285. doi:10.1038/s41467-020-16164-1
53. Monteran L, Erez N. The dark side of fibroblasts: cancer-associated fibroblasts as mediators of immunosuppression in the tumor microenvironment. *Front Immunol*. 2019;10. doi:10.3389/fimmu.2019.01835
54. Dvorak HF. Tumors: wounds that do not heal. *N Engl J Med*. 1986;315:1650–1659. doi:10.1056/NEJM198612253152606
55. Calon A, Lonardo E, Berenguer-Llergo A, et al. Stromal gene expression defines poor-prognosis subtypes in colorectal cancer. *Nat Genet*. 2015;47 (4):320–329. doi:10.1038/ng.3225
56. Hesketh M, Sahin KB, West ZE, Murray RZ. Macrophage phenotypes regulate scar formation and chronic wound healing. *Int J Mol Sci*. 2017;18(7):1545.
57. Lee B, Lee SH, Shin K. Crosstalk between fibroblasts and T cells in immune networks. *Front Immunol*. 2023;13:1103823. doi:10.3389/fimmu.2022.1103823
58. Nieman KM, Romero IL, Van Houten B, Lengyel E. Adipose tissue and adipocytes support tumorigenesis and metastasis. *Biochim Biophys Acta Mol Cell Biol Lipids*. 2013;1831(10):1533–1541. doi:10.1016/j.bbalip.2013.02.010
59. Duman C, Yaqubi K, Hoffmann A, et al. Acyl-CoA-Binding protein drives glioblastoma tumorigenesis by sustaining fatty acid oxidation. *Cell Metab*. 2019;30(2):274–289.e5. doi:10.1016/j.cmet.2019.04.004
60. Kahn CR, Wang G, Lee KY. Altered adipose tissue and adipocyte function in the pathogenesis of metabolic syndrome. *J Clin Invest*. 2019;129 (10):3990–4000. doi:10.1172/JCI129187
61. Crewe C, An YA, Scherer PE. The ominous triad of adipose tissue dysfunction: inflammation, fibrosis, and impaired angiogenesis. *J Clin Invest*. 2017;127(1):74–82. doi:10.1172/JCI88883
62. Mukherjee A, Bilecz AJ, Lengyel E. The adipocyte microenvironment and cancer. *Cancer Metastasis Rev*. 2022;41(3):575–587. doi:10.1007/s10555-022-10059-x
63. Lei X, Lei Y, Li JK, et al. Immune cells within the tumor microenvironment: biological functions and roles in cancer immunotherapy. *Cancer Lett*. 2020;470:126–133. doi:10.1016/j.canlet.2019.11.009

64. Voskoboinik I, Smyth MJ, Trapani JA. Perforin-mediated target-cell death and immune homeostasis. *Nat Rev Immunol*. 2006;6(12):940–952.
65. Aras S, Zaidi MR. TAMeless traitors: macrophages in cancer progression and metastasis. *Br J Cancer*. 2017;117(11):1583–1591. doi:10.1038/bjc.2017.356
66. Narayanan S, Kawaguchi T, Peng X, et al. Tumor infiltrating lymphocytes and macrophages improve survival in microsatellite unstable colorectal cancer. *Sci Rep*. 2019;9(1):13455. doi:10.1038/s41598-019-49878-4
67. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol*. 2013;13(3):159–175.
68. Thiery J, Lieberman J. Perforin: a key pore-forming protein for immune control of viruses and cancer. *Subcell Biochem*. 2014;80:197–220. doi:10.1007/978-94-017-8881-6_10
69. Bied M, Ho WW, Ginhoux F, Blériot C. Roles of macrophages in tumor development: a spatiotemporal perspective. *Cell Mol Immunol*. 2023;20(9):983–992.
70. Bevins NJ, Okamura R, Montesion M, Adashek JJ, Goodman AM, Kurzrock R. Tumor infiltrating lymphocyte expression of PD-1 predicts response to Anti-PD-1/PD-L1 immunotherapy. *J Immunother Precis Oncol*. 2022;5(4):90–97. doi:10.36401/JIPO-22-9
71. Kim JM, Rasmussen JP, Rudensky AY. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat Immunol*. 2007;8(2):191–197. doi:10.1038/ni1428
72. Wolf D, Sopper S, Pircher A, Gastl G, Wolf AM. Treg(s) in cancer: friends or Foe? *J Cell Physiol*. 2015;230(11):2598–2605. doi:10.1002/jcp.25016
73. Ebner DK, Tinganelli W, Helm A, et al. The immunoregulatory potential of particle radiation in cancer therapy. *Front Immunol*. 2017;8. doi:10.3389/fimmu.2017.00099
74. Goodman LS, Wintrobe MM, Dameshek W, et al. NITROGEN MUSTARD THERAPY: use of Methyl-Bis(Beta-Chloroethyl)amine hydrochloride and Tris(Beta-Chloroethyl)amine hydrochloride for Hodgkin's disease, lymphosarcoma, leukemia and certain allied and miscellaneous disorders. *JAMA*. 1984;251:2255. doi:10.1001/jama.1984.03340410063036
75. Guillerey C, Huntington ND, Smyth MJ. Targeting natural killer cells in cancer immunotherapy. *Nat Immunol*. 2016;17(9):1025–1036. doi:10.1038/ni.3518
76. Tsou P, Katayama H, Ostrin EJ, Hanash SM. The emerging role of B cells in tumor immunity. *Cancer Res*. 2016;76(19):5597–5601. doi:10.1158/0008-5472.CAN-16-0431
77. Zinzani PL, Minotti G. Anti-CD19 monoclonal antibodies for the treatment of relapsed or refractory B-cell malignancies: a narrative review with focus on diffuse large B-cell lymphoma. *J Cancer Res Clin Oncol*. 2022;148(1):177–190. doi:10.1007/s00432-021-03833-x
78. Van Coillie S, Wiernicki B, Xu J. Molecular and cellular functions of CTLA-4. In: Xu J, editor. *Regulation of Cancer Immune Checkpoints: Molecular and Cellular Mechanisms and Therapy*. Springer; 2020:7–32. doi:10.1007/978-981-15-3266-5_2
79. Kebriaei P, Singh H, Huls MH, et al. Phase I trials using *Sleeping Beauty* to generate CD19-specific CAR T cells. *J Clin Invest*. 2016;126(9):3363–3376. doi:10.1172/JCI86721
80. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J*. 2021;11(4):1–11. doi:10.1038/s41408-021-00459-7
81. Commissioner O of the. U.S. Food and Drug Administration. FDA; 2024. Available from: https://www.fda.gov/. Accessed August 9, 2024.
82. Gross JC, Chaudhary V, Bartscherer K, Boutros M. Active Wnt proteins are secreted on exosomes. *Nat Cell Biol*. 2012;14(10):1036–1045. doi:10.1038/ncb2574
83. Polanco JC, Li C, Bodea LG, Martinez-Marmol R, Meunier FA, Götz J. Amyloid-β and tau complexity — towards improved biomarkers and targeted therapies. *Nat Rev Neurol*. 2018;14(1):22–39. doi:10.1038/nrneurol.2017.162
84. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol*. 2002;2(8):569–579. doi:10.1038/nri855
85. Urabe F, Kosaka N, Ito K, Kimura T, Egawa S, Ochiya T. Extracellular vesicles as biomarkers and therapeutic targets for cancer. *Am J Physiol Cell Physiol*. 2020;318(1):C29–C39. doi:10.1152/ajpcell.00280.2019
86. Bebelman MP, Smit MJ, Pegtel DM, Baglio SR. Biogenesis and function of extracellular vesicles in cancer. *Pharmacol Ther*. 2018;188:1–11. doi:10.1016/j.pharmthera.2018.02.013
87. Al-Nedawi K, Meehan B, Rak J. Microvesicles: messengers and mediators of tumor progression. *Cell Cycle*. 2009;8(13):2014–2018. doi:10.4161/cc.8.13.8988
88. Weber CE, Kothari AN, Wai PY, et al. Osteopontin mediates an MZF1–TGF-β1-dependent transformation of mesenchymal stem cells into cancer-associated fibroblasts in breast cancer. *Oncogene*. 2015;34(37):4821–4833. doi:10.1038/onc.2014.410
89. Li L, Li C, Wang S, et al. Exosomes derived from hypoxic oral squamous cell carcinoma cells deliver miR-21 to normoxic cells to elicit a prometastatic phenotype. *Cancer Res*. 2016;76(7):1770–1780.
90. Sutherland TE, Dyer DP, Allen JE. The extracellular matrix and the immune system: a mutually dependent relationship. *Science*. 2023;379(6633):eabp8964. doi:10.1126/science.abp8964
91. Winkler J, Abisoye-Ogunniyan A, Metcalf KJ, Werb Z. Concepts of extracellular matrix remodelling in tumour progression and metastasis. *Nat Commun*. 2020;11(1):5120. doi:10.1038/s41467-020-18794-x
92. Herrmann IK, Wood MJA, Fuhrmann G. Extracellular vesicles as a next-generation drug delivery platform. *Nat Nanotechnol*. 2021;16(7):748–759. doi:10.1038/s41565-021-00931-2
93. Aaltonen LA, Abascal F, Abeshouse A, et al. Pan-cancer analysis of whole genomes. *Nature*. 2020;578(7793):82–93. doi:10.1038/s41586-020-1969-6
94. Hoadley KA, Yau C, Hinoue T, et al. Cell-of-origin patterns dominate the molecular classification of 10,000 tumors from 33 types of cancer. *Cell*. 2018;173(2):291–304.e6. doi:10.1016/j.cell.2018.03.022
95. Sanchez-Vega F, Mina M, Armenia J, et al. Oncogenic signaling pathways in the cancer genome atlas. *Cell*. 2018;173(2):321–337.e10. doi:10.1016/j.cell.2018.03.035
96. Thorsson V, Gibbs DL, Brown SD, et al. The immune landscape of cancer. *Immunity*. 2018;48(4):812–830.e14. doi:10.1016/j.immuni.2018.03.023
97. Nofech-Mozes I, Soave D, Awadalla P, Abelson S. Pan-cancer classification of single cells in the tumour microenvironment. *Nat Commun*. 2023;14(1):1615. doi:10.1038/s41467-023-37353-8
98. OPAS/OMS. Organização Pan-Americana da Saúde. Available from: https://www.paho.org/pt. Accessed August 9, 2024.