Mapping the Cell-Membrane Proteome to the Cancer Hallmarks

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Key words: cell-membrane, proteome, cancer hallmarks

Abstract

The hallmarks of biological processes that underlie the development of cancer have been long recognized, yet, existing therapeutic treatments cannot prevent cancer from continuing to be one of the leading causes of death worldwide. This work was aimed at exploring the extent to which the cell-membrane proteins are implicated in triggering cancer hallmark processes. To achieve this goal, we created a database of human proteins associated broadly with ten cancer hallmarks, and mapped the cell-membrane proteome of the SKBR3/HER2+ breast cancer cell line to the hallmark categories. We show that the landscape of the cancer cell-membrane proteome points to unique opportunities that can be exploited to guide novel therapeutic interventions, including multi-agent, immuno-oncology and precision medicine applications.

Significance. Through heavy involvement in supporting cancer hallmark processes, the membrane proteome reveals an extensive catalogue of functionally diverse and networked proteins expected to inspire new cancer therapeutic perspectives.

Introduction

The cancer hallmarks were first described by Hanahan and Weinberg [1,2], and evolved to include six fundamental biological capabilities that sustain neoplastic growth (sustained proliferative signaling, insensitivity to anti-growth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis), two emerging hallmarks of a more general nature (reprogramming of energy metabolism and evasion of immune destruction), and two enabling characteristics (genome instability and inflammation). Genetic changes drive the formation of a primary tumor, however, cell-autonomous mechanisms propelled by a diverse mutational landscape are not sufficient to explain the full range of aberrant behaviors. For example, tissue invasion and metastatic propensity have been described as being driven by a supportive tumor micro- or systemic macro-environment and epigenetic re-programming rather than metastasis-specific driver mutations [3-7]. Mutations, on the other hand, have been shown to heavily affect epigenetic regulators [8]. Also, synergistic effects enabled by cell-extrinsic environmental stimuli (various signaling, growth, cytokine or angiogenic factors, recruited stromal cells, etc.) and cell-intrinsic EMT gene regulators have been proposed to drive the metastatic processes via adaptation rather than selection [8-11]. As a result, in support of the metastatic process, additional hallmarks that include motility/invasion, microenvironment modulation, plasticity, and colonization have been proposed [12]. Moreover, the presence of a tumor microbiome has been implicated in tumor-supportive inflammation and tumor progression [13]. Altogether, genetic and epigenetic alterations work in tandem with a cooperative environment to reprogram gene expression, corrupt biological regulatory pathways, mediate adaptation in support of malignant neoplastic growth and metastasis, and ultimately drive the evolution of drug resistance [8,14]. Such non-cell-autonomous mechanisms act through the interface between the cancer cells and their environment. This interface is defined by the cell-membrane that imbeds proteins with critical roles in intra-cellular signaling and inter-cellular communication, cell-cell, cell-pathogen and immune recognition events, cell-adhesion and motility, and exchange of solutes

and various factors. Cell-surface receptor tyrosine kinases (RTKs) trigger vital signaling cascades that control proliferation, differentiation, growth and metabolism. RTKs initiate the signaling process and regulate intracellular events by binding various ligands such as growth factors, peptides and hormones. Aberrant or mutated expression of such receptors is often the driver of uncontrolled proliferation. In contrast, cytokine receptors initiate signaling processes through association with other non-receptor protein kinases, while membrane proteases support secretory, cell-cell signaling, and degradation functions by cleaving the membrane-bound proteins. Plasma membrane proteins also interact with lipids and carbohydrates to sustain various transport and endo-/exocytic processes to ensure in-and-out shuffling of solutes, nutrients, hormones, growth factors, cytokines, and other signaling and ECM molecules. Cell-junction and adhesion proteins, on the other hand, are critical not just to determining the 3D architecture of cell conglomerates, but also to inter-cellular or cell-ECM communication, as well as functionality within a tissue. In this work, SKBR3/HER2+ cells were used as a model system to explore the cancer hallmarks through the perspective of the cell-membrane proteome. Proteomic datasets generated from these cells by complementary methods were analyzed and queried for the presence of proteins that could be correlated with hallmark processes. The results are assessed in the context of emerging interest in novel therapeutic paradigms that seek a systems approach for identifying drug target combinations with synergistic effects.

Methods

Proteomic LC-MS/MS raw files from the PRIDE Archive (see below access info) and associated protein lists [15] were used in this study to map detectable membrane proteins in the SKBR3 breast cancer cell line to cancer hallmark processes. A detailed description of the experimental conditions for cell culture, cell-membrane protein labeling/isolation, MS analysis, and annotation of membrane proteins is provided in reference [15]. Briefly, SKBR3 cells were acquired from ATCC (Manassas, VA), authenticated by STR (ATCC), grown in serum-free and serum-rich culture media, and processed for generating cell-membrane enriched protein fractions. The SKBR3 cell-membrane proteins were isolated by either the biotinylation of amino or glycan groups followed by affinity NeutrAvidin pulldown, or by tryptic shaving of cell-surface proteins in culture. Three biological replicates of cells cultured in the presence or absence of serum, enriched in membrane proteins according to all three protocols, were generated. The proteolytic digests of the isolated proteins from all biological cell states and replicates were analyzed in triplicate by mass spectrometry using an EASY-nLC 1200 UHPLC/QE-Orbitrap-MS system (Thermo Fisher Scientific).

The mass spectrometry data were analyzed by using the ProteomeDiscoverer 2.4 software package (Thermo Fisher Scientific), the Sequest HT search engine, and a reviewed, minimally redundant *Homo sapiens* database from UniProt with 20,433 entries (2019 download) [16]. The quality of protein IDs that were mapped to the hallmark supportive database was determined by: (a) the stringency of peptide assignments as defined by parent/fragment ion mass tolerances of 15 ppm and 0.02 Da, respectively; (b) the use of only rank 1 peptides and top scoring proteins; and (c) the use of a minimum number of two unique peptide matches to a protein sequence. All FDR targets for peptide spectrum matches, peptide and protein groups were set to either 0.03 (relaxed) or 0.01 (strict), and protein grouping was accomplished by using the strict parsimony principle. Protein abundance was provided as log10(protein spectrum counts), i.e., log10 (SC). Protein assignments

to the cell-membrane or cell surface were made by using UniProt/GO annotations, the Human Protein Atlas cellular and organelle proteome database, and literature reports [15-18].

The database (**Table 1**) defining the biological processes that support the cancer hallmarks was constructed by extracting the relevant biological processes and pathways from the UniProt *Homo sapiens* database using controlled vocabulary GO annotation terms [17]. Only reviewed protein entries that were associated with these processes in the period July 2020-June 2021 were included in the lists. The COSMIC (v94) Cancer Gene Census catalogue (CGC) [19], along with other hallmark proteins reported in the literature [1, 2, 20-24], were used for a curated definition of cell-membrane hallmark proteins. The circular data plot was created using the Galaxy platform [25] and the Circos data visualizing package [26]. Protein-protein interaction networks, with interactions scores set to high, were created in STRING [27] and visualized with Cytoscape tools [28]. This work did not involve the use of human subjects, and did not require IRB approval.

Data availability. Supplemental table 1, comprising the protein IDs associated with the cell-membrane cancer hallmark processes, and Supplemental figure 1 providing the extended PPI networks are available on the journal website. This study used mass spectrometry raw files from the ProteomeXchange Consortium via the PRIDE partner repository with the following dataset identifiers: PXD028976, PXD028977, and PXD028978.

Results

Given the critical role of the plasma membrane in determining the fate of a cell, we hypothesized that many proteins that are integral to- or associated with the plasma membrane can be re-evaluated in the context of cancer hallmarks to support the detection and therapeutic treatment of cancerous cell states. To this end, an in-house database (DB) comprising ten broad categories of proteins that are either actively participating in-, or are just supportive or enablers of the cancer hallmarks was created by using the UniProt *Homo sapiens* DB and GO biological process and pathway annotations. **Table 1** provides the original hallmark categories, the biological processes and pathways associated with the specific hallmarks, and the number of proteins matched to each category. Overall, 6,258 cell-membrane/cell-surface proteins, were associated with the hallmark DB (**Figure 1A** and **Supplemental table 1**). The number of experimentally detected cell-membrane proteins, and examples of cell-membrane hallmark proteins described in the literature [20-24] or reported in the CGC DB with cancer promoting, suppressing or dual role [19], are also provided. Decision for associating a particular biological process or pathway with a hallmark was made based on relevance and availability of adequate GO annotations.

To test our hypothesis, a dataset of SKBR3 proteins generated by various experimental cell-surface enrichment processes [15] was queried for matches to the cancer hallmark DB. From the reported number of 1,316 cell-membrane and surface proteins identified by mass spectrometry (MS) with high confidence [15], as many as 1,127 could be matched to multiple hallmarks (**Table 1** and **Supplemental table 1**). A Circos plot with radially distributed hallmarks, in which each cell-membrane protein was mapped to its corresponding gene locus within the 23 human chromosomes, highlights the complex genetic landscape that can lead to cancer through various combinations of genes with altered functional products (**Figure 1B**). The cell-membrane proteins represent ~35-40 % of the encoded proteins by each chromosome, with ~5 % being detectable in SKBR3 when using

the described experimental conditions (**Figures 1C&D**). The hallmark-supportive proteins were encoded by the entire genome, excepting chromosome Y which did not have any matches because the SKBR3 cells originate from a female subject. Protein abundance was represented by scatter plots depicting the log10(protein spectrum counts), with dot size representing a range spanning from 0 to 5. The non-coding centromeric areas and acrocentric chromosomal p-arms (13, 14, 15, 21, and 22) did not display any products (**Figures 2A**&**B**), while some chromosomal coding regions appeared to be either under- (4p/4q, 6p, 14q, 17p, Xp, 18q, and 22q) or over-represented (21q) experimentally (**Figures 2C**&**D**). The 14 proteins encoded by 21q were indicative of positive regulation of ROS metabolic and immune system processes, whereas angiogenic processes emerged more prominently across the entire spectrum as being supported by a range of receptors and adhesion proteins (e.g., THBS1, FGFR1/2, NOTCH1/2, TGFBR1, ERBB, CDC42, CAMs, ephrins, and integrins). The interpretation of such data calls, however, for prudence and further validation, as the SKBR3 cell line is highly aneuploid with numerous structural and numerical chromosomal aberrations (modal chromosome number 84), and the mechanistic impact of chromosomal alterations on protein expression and pathogenic outcomes still lacks a thorough understanding.

To corroborate the relevance of these cell-membrane proteins to cancer, the COSMIC CGC list was queried against the hallmark database (**Table 1** and **Supplemental table 1**). The CGC comprises evidence-based, manually-curated information related to over 700 cancer-driving genes [19]. The majority of CGC proteins (621) could be mapped to the hallmark database, encompassing 91 experimentally identified cell-membrane proteins of which 74 were tier 1 (i.e., with documented cancer-relevant activity and evidence of mutations that support oncogenic transformation) and 17 were tier 2 (i.e., with strong indications of cancer-related activity, but lack of sufficient evidence). The detected CGC cell-membrane subset comprised 44 oncogenes and 28 tumor suppressors, several with overlapping function. Overall, based on literature reports [1,2,19-24], 116 of the detected proteins were catalogued with hallmark roles, of which 48 were also logged by the CGC. Not surprisingly, the top Kegg pathways that could be associated with these proteins included cancer-enabling (PI3K/AKT, MAPK, Rap1, RAS, EGFR tyrosine kinase inhibitor resistance, signaling regulating pluripotency of stem cells) and migration supportive pathways (regulation of actin cytoskeleton, adherence junction, ECM-receptor interaction, focal adhesion). Many small molecule or monoclonal antibody drugs matched to these proteins, in various stages of development, have been already listed in the DrugBank database (**Figure 3**). Protein-protein interaction (PPI) networks constructed based on the 116 documented hallmark proteins (**Figure 4**), or based on the whole set of 1,127 proteins (**Supplemental figure 1**), underscored the intimate involvement of the SKBR3 cell-membrane proteome in the progression of cancer.

Discussion

All major cell-membrane protein categories, i.e., with receptor/catalytic activity, adhesion/junction, transport, and CD classification, were represented in every cancer hallmark. The hallmarks of cell communication/signaling, adhesion/motility, immune response and cell cycle/growth accrued the largest number of protein hits, reflecting their considerable impact on cancer development and dissemination (**Table 1**). Some of the highest abundance membrane

proteins were part of the very same categories, and included receptors, adhesion proteins, solute carriers, and transporters, as well as additional ephrins, nectins, integrins and essentially all detected CDs. A few specific examples, that also comprise hallmark proteins from the classic literature (highlighted in bold), include members with multiple roles in receptor mediated signaling (**ERBB2/CD340**, **EGFR**, TFRC/CD71, PTPRF, ITGAV/CD51, ITGB1, BCAM, PVR, SCARB1, SUSD2), adhesion (ITGAV, ITGB1/CD29, BCAM, **EPCAM/CD326**, PVR, SCARB1, MUC16, PVR, PTPRF, **EGFR**, **ATP1B1**, SCARB1), immune response (SUSD2, PVR, SCARB1, ITGAV, **EGFR**, TFRC/CD71, CD44), and transport (ABCC1, ATP1A1, **ATP1B1**).

The PPI networks exposed highly interconnected protein clusters with several members playing roles in multiple hallmark categories (e.g., EGFR, ERBB2, FGFRs, CD44, SRC, STAT3). The emerging information can be used to identify novel regulatory or effector proteins, targets for knockout experiments, components of protein complexes, or to assign functionality to yet uncharacterized proteins. Through PPI networks, attention can be expanded to a vast set of membrane proteins that when targeted in a combinatorial or sequential fashion can impair cancer proliferation and dissemination not only through well-established mechanisms such as RAS/ERK or PI3K/AKT signaling, but also via processes implicated in angiogenesis, migration, cell death, metabolic reprogramming, inflammation, or remodeling of the tumor microenvironment. The ability to identify by MS aberrant protein isoforms or posttranslational modification patterns (e.g., glycosylation in the case of cancers [29]) can further strengthen the efficacy of this approach. Pathways that confer intrinsic or acquired drug resistance and that often work via cross-talk or transactivation, or that lead to cancer stem cell mediated resistance, have been of particular interest to drug developers. Informed selection of drug targets, rather than based on empirical screening, will better suppress redundant or compensatory signaling pathways, prevent tumor-induced vascular remodeling, disable the ECM supportive environment, overturn immune evasion capabilities, and impair metastatic potential.

The SKBR3 cell-membrane landscape of oncogenes, tumor suppressors and existing drug targets reveals one example of a network of key receptors (EGFR, ERBB, FGFR, IGFR, MET, MTOR, MAPK/RAS proteins), CD antigens (CD274, CD44), adhesion proteins (EPCAM, MUC1), and ABC transporters (ABCC1/2/3) that have been already explored for effective targeting of various cancers (**Figure 3**). Such complex protein signatures and network architectures enable the targeting of broad panels of interconnected RTKs or CDs, and can uncover vulnerabilities that may trigger cascading failures from either within the established (**Figure 4**) or the extended networks (**Supplemental figure 1**). This, in turn, can enable combating widespread strategies used by cancer cells to resist the action of drugs, such as MET amplification or PI3K/AKT activation after targeted EGFR inhibition, PI3K/AKT compensation after mTOR inhibition, or MAPK/STAT3 cross-talk [14]. Targeting orthogonal pathways by using combinations of signal transduction or angiogenesis inhibitors, inducers of apoptosis, and inhibitors of drug efflux proteins (ABCC1 or multidrug resistance associated protein-MRP1), can be further explored to selectively attack or eliminate the cancer cells. Moreover, tumor-stroma interactions that support cancer progression and metastasis can be interrupted by disabling paracrine (FGF, Wnt, Hedgehog, TGFβ, NOTCH) and autocrine signaling sustained by cell-membrane receptors and proteases. Therapies aimed at modulating the tumor-immune cell dialogue, by using for example check point inhibitors such as the anti-programmed cell death ligand 1 (PD-L1 or CD274)/anti-PD1, are expected to find a fertile ground in the rich backdrop of cell-membrane antigens. Such immunotherapies can be used alone or jointly with chemo- or targeted therapies.

In summary, mapping the SKBR3 cell-membrane proteome to cancer hallmark-supportive processes exposed a complex scenery of critical players that promote and sustain the development and metastatic propensity of these cancer cells. The cell-membrane harbors an abundant supply of putative targets for the development of modern cancer therapies, future work being needed to refine the list of viable candidates. With the advance of high-throughput MS instrumentation, the ability to quickly generate tumor cell-membrane profiles will create the necessary resource for exploring complex and aberrant protein expression signatures, studying additive or synergistic effects of drug combinations that target hallmark proteins, understanding the evolution of (multi)drug resistance, evaluating cancer risk and prognostic factors, and facilitating the selection of patient-tailored drug targets for achieving superior therapeutic outcomes in the various stages of cancer development.

Acknowledgment. This work was supported by an award from the National Institute of General Medical Sciences to IML (R01 Grant No. GM121920).

Author contributions. AK processed and analyzed the mass spectrometry data, reviewed and queried the literature for cancer hallmark proteins, and constructed the PPI networks. JRSH analyzed the hallmark protein dataset and generated the Circos plot. IML conceptualized the work, built the cancer hallmark protein database, and wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

Conflict of interest disclosure. The authors declare no conflict of interest.

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Table 1. Cancer hallmarks defined by GO biological processes and examples of cell-membrane proteins associated with the hallmarks. Proteins catalogued in the CGC have promoting, suppressing, or dual role.

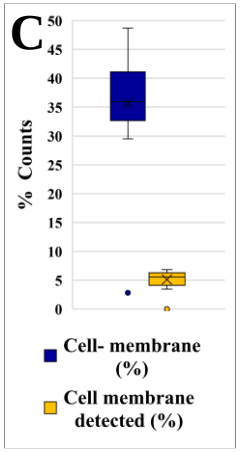
Cancer hallmarks [1,2]	**Biological processes associated with the cancer hallmarks based on UniProt/GO annotations**	**Protein IDs -Total/genome -Cell-membrane -Detected**	**Hallmark proteins associated with the cell membrane [1,2,14-19]**	**Cell membrane proteins present in the CGC with cancer promoting (↑), suppressing (↓), or dual role (↑↓).**
Sustained proliferative signaling	• Cell communication (& regulation) • Signaling (& regulation) • Signaling receptor activity (& regulation)	6871 4058 669	RRAS, NRAS, HRAS, KRAS, EGFR, ERBB2, FGFR1/2, MAP2K1/2, MTOR, MET, IGF1R, ***ABCC1***	NRAS ↑, HRAS ↑, RAC1 ↑, CALR ↑, ACVR1 ↑, DNM2 ↑, EPS15 ↑, ERBB3 ↑, ERBB4 ↑, ERBB2 ↑, EZR ↑, FGFR3 ↑, FGFR1 ↑, NDRG1 ↑, NOTCH1 ↑, NOTCH2 ↑, EGFR ↑, IL6ST ↑, KRAS ↑, PRKAR1A ↑, GNA11 ↑, GNAQ ↑, GNAS ↑, JAK1 ↑, MTOR ↑, MET ↑, FGFR2 ↑, FGFR4 ↑, MYD88 ↑
Insensitivity to anti-growth signals (evading growth suppressors)	• Cell cycle, cell division, cell growth (& regulation) • Cell population proliferation (& regulation) • Protein catabolic process (& regulation) • Protein folding (& regulation) • Protein targeting (& regulation) • Signaling pathways & their regulation (PI3K, TOR, Wnt, signal transduction by p53 class mediator)	4925 1951 500		RHOA ↑, ATP2B3 ↑, DDX3X ↑, ERBB4 ↑, FAT1 ↑, NDRG1 ↑, NOTCH2 ↑, GNAS ↑
Evasion of immune destruction	• Immune system process • Innate and adaptive immune response (& regulation) • Antigen processing and presentation (& regulation) • Autophagy (& regulation) • Cellular senescence • Cellular response to hypoxia (& regulation) • Chemotaxis (& regulation) • Chemokine mediated signaling pathway (& regulation) • Chemokine production (& regulation) • (Myeloid/B-cell/T-cell activation and regulation are included)	4055 2272 532	CD274, ***SIGLEC6***	NRAS ↓, RAC1 ↑, B2M ↓, EGFR ↑, JAK1 ↓, MET ↑
Limitless replicative potential	• DNA replication (& regulation) • Chromosome organization (& regulation) • Signal transduction by p53 class mediator (& regulation) • Telomere maintenance (& regulation)	1497 251 71	***RAP1A***, ***ITGB1***, ***TPP1***	NRAS ↑, FGFR1 ↑, NDRG1 ↓, NOTCH1 ↑, KRAS ↑
Inflammation	• Inflammatory response (& regulation) • NFKB signaling (& regulation)	2391 1323 280	COL1A1, ***DDR1***, ABCC1/2/***3***	RHOA ↓, NRAS ↑, IL6ST ↑, KRAS ↑, MYD88 ↑

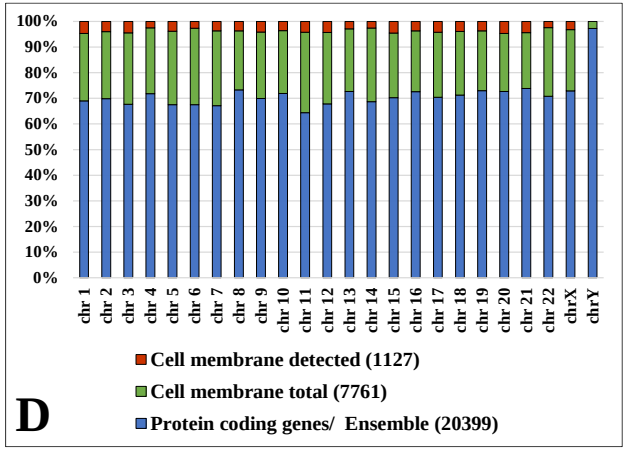
	• I-kappaB kinase/NF-kappaB signaling (& regulation) • Receptor signaling pathw. via STAT (& regulation) • MAPK cascade (& regulation) • TNF mediated signaling pathway (& regulation) • Macrophage activation (& regulation) • Cellular response to cytokine stimulus (& regulation)			
Tissue invasion and metastasis	• Cell adhesion (& regulation) • Cell motility (& regulation) • Actin cytoskeleton organization (& regulation) • ECM organization (& regulation) • Secretion • EMT (& regulation)	4650 2646 593	CD44, EPCAM, POSTN, TNC, LGALS1, ABCC1	RHOA ↑↓, NRAS ↑, NRAS ↑, RAC1 ↑, CALR ↑, ACVR1 ↑, DDX3X ↑↓, ATP1A1 ↑, BMPR1A ↑, DNM2 ↑, ERBB3 ↑, ERBB2 ↑, EZR ↑, FAT1 ↑↓, FGFR1 ↑, FHIT ↓, NDRG1 ↓, NOTCH2 ↑, EGFR ↑, KRAS ↑, PRKAR1A ↓, MTOR ↑, MET ↑, FGFR4 ↑, MYD88 ↑
Sustained angiogenesis	• Angiogenesis (& regulation) • VEGF signaling (& regulation)	504 294 84	***NRAS, HRAS, KRAS***, ***AGRN***, HSPG2, ***SDC1/4***, THBS1, ***LGALS1***	NRAS ↑, RAC1 ↑, CALR ↑, DNM2 ↑, NOTCH1 ↑, EGFR ↑, KRAS ↑, SDHA ↓, MTOR ↑, MET ↑
Genome instability	• Cellular response to DNA damage stimulus (& regulation) • Disease variant	4030 1659 399	COL7A1, FEN1, MSH6	USP8 ↓, RHOA ↓, NRAS ↑, RAC1 ↓, CLTC ↓, FHIT ↓, LMNA ↓, ERCC4 ↑↓, MET ↑
Evasion of apoptosis (resisting cell death)	• Cell death (& regulation) • Cell aging (& regulation) • Autophagy (& regulation) • (Includes apoptosis, senescence, necrosis)	2602 1139 323	ITGB1	RHOA ↑↓, NRAS ↑, NRAS ↑, RAC1 ↑, CALR ↑, ACVR1 ↑, DDX3X ↓, ATP1A1 ↑, ERBB3 ↑, ERBB4 ↑↓, ERBB2 ↑, EZR ↑, FAT1 ↑, FGFR3 ↑, NOTCH1 ↑, NOTCH2 ↑, EGFR ↑, KRAS ↑↓, PRKAR1A ↑↓, JAK1 ↓, MTOR ↑, MET ↑↓, FGFR2 ↑, MYD88 ↑
Deregulating cellular energetics (reprogramming of energy metabolism)	• Gluconeogenesis (& regulation) • Glycolytic process (& regulation) • Canonical glycolysis • Pyruvate oxidation • TCA cycle • Oxidative phosphorylation • Electron transport chain • ATP metabolic process (& regulation) Carbohydrate metabolic process (& regulation) • Lipid metabolic process (& regulation) • One carbon metabolic process • Choline metabolic process • Cellular response to hypoxia (& regulation) • Peroxisome proliferator activated receptor signaling pathway (& regulation) • Energy homeostasis	2300 880 242	***VDAC1***, ATP1B1, GAPDH, IDH2, PFKM, ATP6V1B1, SLC2A1	USP8 ↑, DDX3X ↑, ERBB2 ↑, NOTCH1 ↑, EGFR ↑, PICALM ↑, PAFAH1B2 ↑, KRAS ↑, SDHA ↑, MTOR ↑
Note: according to GO annotations, the proteins highlighted in bold/italic are part of other hallmark categories than reported in the literature.				

Figure 1. Cell-membrane proteins mapped to cancer hallmarks. (**A**) Protein counts with hallmark association. **(B)** Circos plot of detected SKBR3 cell-membrane proteins mapped to their position in the human genome represented by 23 chromosomes, and categorized into 10 radially distributed cancer hallmarks. Hallmark categories, from inside-out: 1-DNA replication, chromosome organization, and telomeres; 2-glycolysis, gluconeogenesis, and carbohydrate metabolism; 3-Angiogenesis; 4-Inflammatory response; 5-Cell death, apoptosis, senescence, aging; 6-Disease mutation; 7-cell cycle division, growth, and proliferation; 8-Immune system processes; 9-Cell adhesion and motility; 10-Cell communication and signaling. The inner PPI network was constructed with proteins for which the STRING interaction score was ≥0.995. (**C**) Percent counts of cell-membrane proteins encoded on, and detected from, each chromosome. (**D**) Distribution of coded (total and cell-membrane) and detected proteins per chromosome.

A

	Total protein IDs	Presence in hallmark DB	Presence in CGC DB	Hallmark & CGC DB
Homo sapiens DB [21]	20,432	13,715	738	621
Hallmark supportive DB	13,715	13,715	621	621
CGS DB [19]	738	621	738	621
Cell-membrane annotation [20]	7761	6258	311	272
Cell-membrane detected [20]	1316	1127	101	91





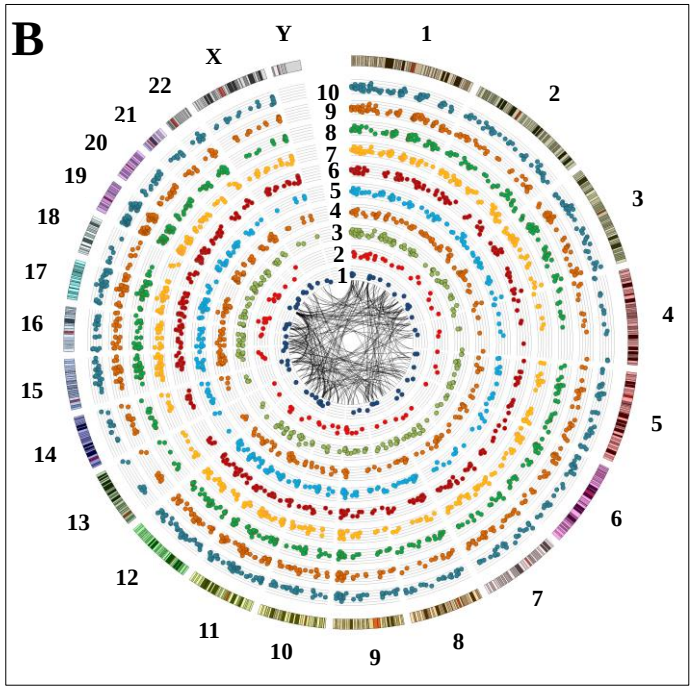


Figure 2. Stacked bar charts representing cancer-supportive human genes and proteins distributed per chromosome arms and per cancer hallmarks; the vertical ordering of hallmarks is following the same numerical trend as in 1B (1-chart bottom, 10-chart top). (**A**) and (**B**) represent % cell-membrane protein-coding genes out of total coding genes per chromosome and p/q arms (the summed percentages exceed 100 because of genes with contributions to multiple hallmarks); (**C**) and (**D**) represent % cell-membrane proteins detected in SKBR3 out of total encoded.

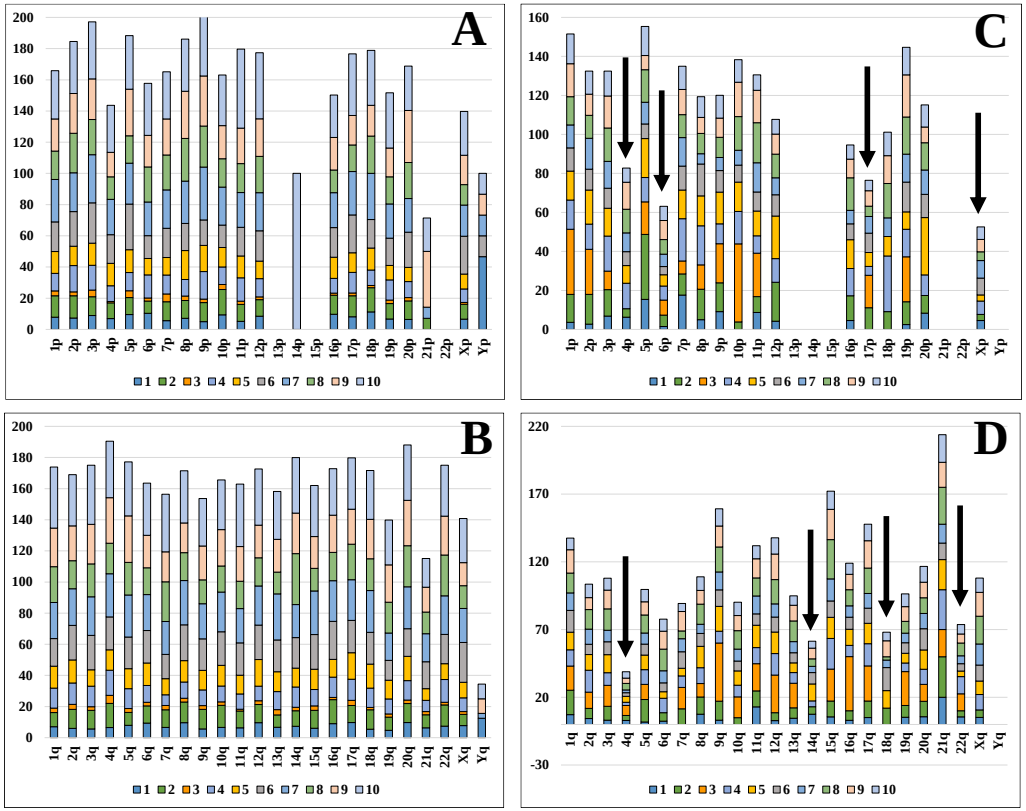


Figure 3. Overlapping proteins between SKBR3 cell-membrane oncogenes, tumor suppressors, and drug targets. Top/left legend indicates the symbols associated with proteins matched to a particular number of hallmarks. Line width indicates the peptide spectral counts matched to each identified protein.

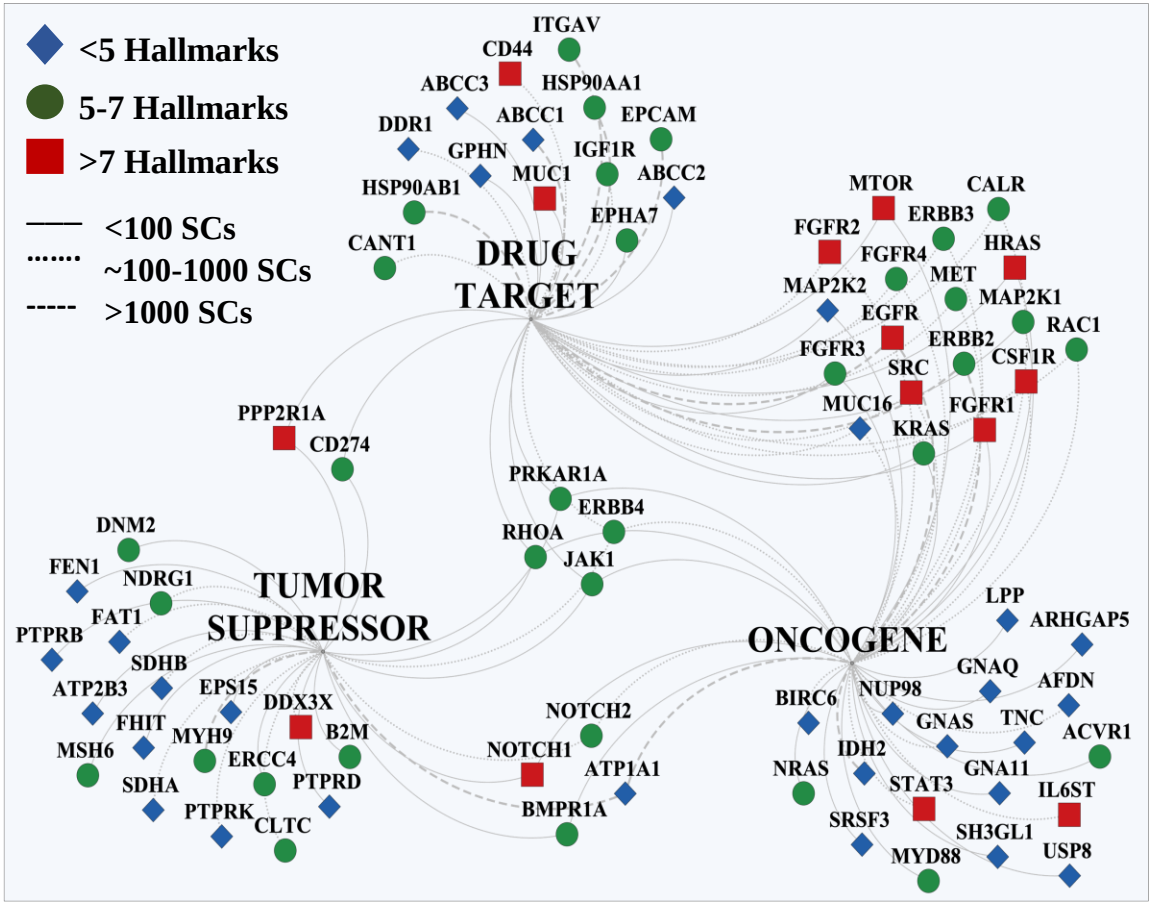
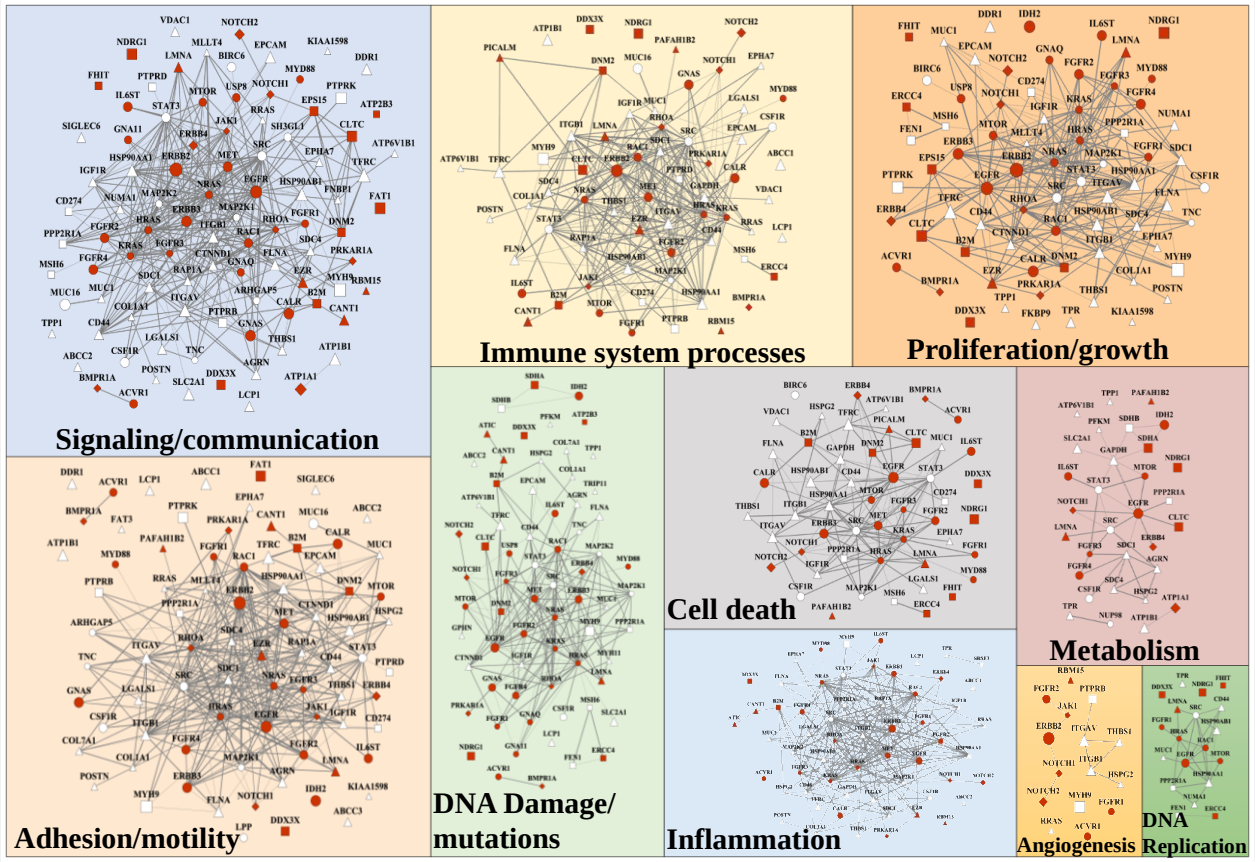



Figure 4. SKBR3 PPI networks constructed from 116 cell-membrane proteins matched to ten cancer hallmarks. CGC proteins are represented as oncogenes (O), suppressors (□), or oncogenes and suppressors (◊); Red icons indicate whether the protein was annotated as a cancer hallmark protein by CGC; Other hallmark proteins (△); Node size is proportional to the protein abundance; Edge thickness reflects the STRING interaction score (set to >0.7/high).