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Toward a genome-based treatment landscape for renal cell carcinoma

Francesco Massari[a,*,1], Vincenzo Di Nunno[a,1], Matteo Santoni[b], Lidia Gatto[a], Claudia Caserta[c], Franco Morelli[d], Elena Zafarana[e], Francesco Carrozza[f], Alessandra Mosca[g], Veronica Mollica[a], Roberto Iacovelli[h], Roberto Sabbatini[i], Camillo Porta[j], Sergio Bracarda[c]

[a] Division of Oncology, S.Orsola-Malpighi Hospital, Bologna, Italy
[b] Macerata Hospital, Macerata, Italy
[c] Medical Oncology, Dept. of Oncology, Azienda Ospedaliera S.Maria, Terni, Italy
[d] U.O.C. Oncologia, Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy
[e] AUSL4 - Nuovo Ospedale di Prato,Prato, Italy
[f] Oncology Unit, City Hospital, Faenza, Italy
[g] S.C. Oncologia Medica, Azienda Ospedaliero-Universitaria Maggiore della Carità, Novara, Italy
[h] U.O.C. Oncologia Medica, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Roma, Italy
[i] Department of Oncology, Hematology & Respiratory Diseases, University of Modena & Reggio Emilia, Modena, Italy
[j] Department of Internal Medicine, University of Pavia and Division of Traslational Oncology, IRCCS Istituti Clinici Scientifici Maugeri, Pavia, Italy

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ABSTRACT

Knowledge about molecular mechanisms driving development and progression of renal cell carcinoma has been elucidated by different studies. In few years we discovered a large difference between genomic landscapes of clear cell and non-clear cell carcinoma. Moreover, tumor heterogeneity and different acquisition of gene mutations during tumor progression are issues of particular interest. In this review we focalized our attention on principal genomic alterations identified among RCC subtypes. Acquired gene mutations may be an adaptive response to several external pressure including metabolic, treatment, genomic and immune-related external pressure. Thus we correlated and discussed principal genomic alterations adopted by tumor to escape from each external pressures. The aim of the present work is to summarize current knowledge about genomic alterations in RCC with special interest of treatment strategies tailored on the basis of disease mutations assessment.

1. Introduction

Renal Cell Carcinoma (RCC), the most frequent renal epithelial tumor, is characterized by distinct histological subtypes. In 2019, over 78,000 new cases of RCC are expected with 14,700 tumor related deaths (Siegel et al., 2019). Clear Cell RCC (ccRCC) accounts for approximately 75–85% of kidney cancer with the remainings classified as "non clear cell Renal Cell Carcinoma" (nccRCC), a broad spectrum of over a dozen of different histopathological entities, according to the 2016 World Health Organization (WHO) Classification. Papillary Renal Cell Carcinoma (pRCC) types 1 and 2 and Chromophobe Renal Cell Carcinoma (chRCC) are the most frequent nccRCC subtypes (10–15% pRCC, 4–5% chRCC) while medullary, translocation, collecting duct, as well as other rarer RCC histotypes represent an infrequent diagnosis (Moch et al., 2016). Each of these tumor subtypes presents a specific and complex spectrum of gene and molecular altered pathways resulting in a heterogeneous mixture of malignancies associated with different morphology, immune-histochemical features, clinical behavior, response to available systemic treatments and prognosis (Giles et al., 2017; Kroeger et al., 2013).

Thanks to recent large genome sequencing studies we have now achieved a better understanding of the genomic issues related to RCC subtypes. In particular, we moved to an extraordinary complex and dynamic scenario in which each RCC tumors type presents a prevalent and specific genetic pathway. As suspected, genomic assessment strongly differs between ccRCC and different nccRCC subtypes (even if shared altered pathways are often reported), with a strong variability of the mutational profile, due to inter- and intra- tumor heterogeneity not only in the same tumor subtypes but also in samples of the same tumor (Ito et al., 2016; Martinez et al., 2013; Brunelli et al., 2007, 2011; Sanfrancesco and Cheng, 2017). As genomic assessment of RCC seems to no longer being a dark matter, next challenge will be represented by

* Corresponding author.
E-mail address: fmassari79@gmail.com (F. Massari).
[1] Equally Contributing First Authors.

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the translation of this knowledge in our clinical practice. In particular, we need to better understand which are the supposed mechanisms hidden behind RCC development and cancerogenesis, as well as the genomic strategies adopted by tumor to sustain its growth. Current picture of genomic landscape of RCC appears extremely complex. However, if we consider which are the external pressure that tumor needs to overtake to sustain its development this complex scenario seems to acquire a defined logic. Recognizing the underlying causes behind the development of some mutations lead us to understand what could happen when we target a specific pathways; of course, this is a key point in order to develop new really tailored drugs and therapeutic strategies. In this review we'll discuss four supposed external pressure such as: metabolic, immune, genomic and treatment related pressure which could trigger some specific mutations pathways sometimes shared by all RCC subtypes which are able to drive tumor development and evolution.

2. TRUNKS, BRANCHES and BRAIDED model

If genomic heterogeneity in RCC is commonly observed and leads to an extraordinary number of possible mutations, ultimately resulting in several different genomic profiles, some shared genomic alterations which have been observed in each tumor subtypes act as driver mutations, providing the bases for eventual tailored approaches.

As an example, in sporadic ccRCC, loss of chromosome 3p is reported in about 91% and mutation of Von Hippel Lindau (*VHL*) gene occurrs in about 54% (+/- 14%) of tumors. On the other hand, recurrent genomic mutations have been observed also in pRCC type I (*MET* mutations), and chRCC (often *PTEN* and *p53* mutated) (Cancer Genome Atlas Research Network, 2013, 2016; Ricketts et al., 2018; Davis et al., 2014).

What has been suggested in RCC is that tumor heterogeneity and genomic profile of each disease strictly depends on specific mutations occurring in different phases of disease development, with also a possible different spatial location. These mutations are often truncal type events (e.g. chromosomal 3 losses in ccRCC) which, once happened, leads to a wide spectrum of mutations which could occurs during tumor progression.

In this model ''trunks'' mutations are represented from generally truncal type events while ''branches'' are the possible mutations resulting from these events. The ''braided'' model also hypothesizes that heterogeneous mutations may happen at different points in time, but the overall genomic profile inevitably becomes similar (Hsieh et al., 2016, 2017a).

An example of this model could be observed in ccRCC. In a study of four patients, *VHL* mutation and 3p loss of heterozygosity were found in all the regions of tumor samples (trunk), while other common mutations recognized as driver mutations (*SETD2, PBRM1, MTOR, BAP1, KDM5C, TSC1.*) were present heterogeneously (branches). These last mutations occurred in specific regions across primary tumor or metastatic samples, possibly reflecting a genomic response to different external pressure which may occur in different specific tumor regions (Gerlinger et al., 2012; Wei and Hsieh, 2015a).

Several studies evaluated genomic expression of RCC providing important issue about genomic landscape of the disease (Cancer Genome Atlas Research Network, 2013, 2016; Ricketts et al., 2018; Davis et al., 2014; Hsieh et al., 2017a; Shuch et al., 2015; Fiorentino et al., 2017). What is emerging from these studies is that each tumor type presents specific chromosome rearrangements and a specific genomic mutational profile:

Chromosome rearrangements (Fig. 2):

- ccRCC: 3p loss (91%), 14q loss (45%), gains of 5q (67%).
- Type I pRCC: gains of chromosomes 7 (75–80%), 16 (60%) and 17 (80%)
- Type II pRCC: 3p and 14p loss (20%), gains of 5q (20%), 7–16 (30%) 17 (20%), 9p loss, and 22q loss (30%)
- chRCC: chromosome 1,261,013 and 17 loss (86%), chromosome 3, 5,891,118 and 21 loss (12–58%).
- MiT family translocation: transocation involving Xp11.23, 6p21
- Collecting duct: loss of 8p,16p,1p and 9p, gains at 13q
- Medullary renal cell carcinoma: chromosome 22.
- Oncocytoma: loss of 1 and y, CCND1 rearrangement

Genomic Mutational Profiles (Cancer Genome Atlas Research Network, 2013, 2016; Ricketts et al., 2018; Davis et al., 2014; Hsieh et al., 2017a; Shuch et al., 2015):

- ccRCC: *VHL, PBRM1, SETD2, KDM5C, PTEN, BAP1, MTOR and P53*.
- pRCC: *MET* (Type I), *SETD2* (type II), *BAP1* (type II), *PBRM1* (type II), *NF2* (type II), CDKN2A (type II), *KDM6A, SMARCB1, FAT1, STAG2, NFE2L2, TP53*.
- ChRCC: TP53, PTEN, MTOR, NRAS, TSC1, TSC2.
- Medullary: *SMARCB1* (*INI-1).*
- Oncocytoma: Mitochondrial genes (*COX1,COX2, MTND4, MTCYB*).

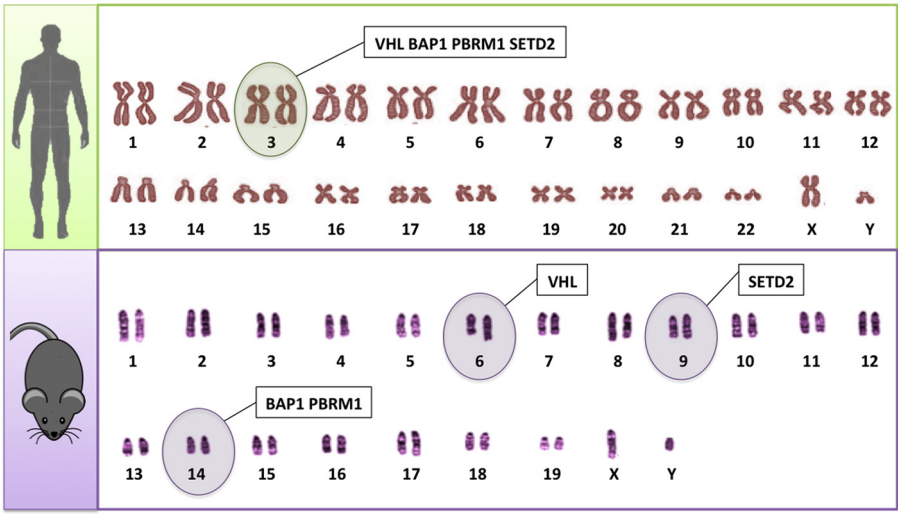

Fig. 1. Chromosome containing principal altered genes in Renal Cell Carcinoma in humans and mice. BRCA 1 associated protein 1 (BAP1), Polybromo 1(PBRM1), SET Domain containing 2 (SETD2), Von Hippel Lindau (VHL).

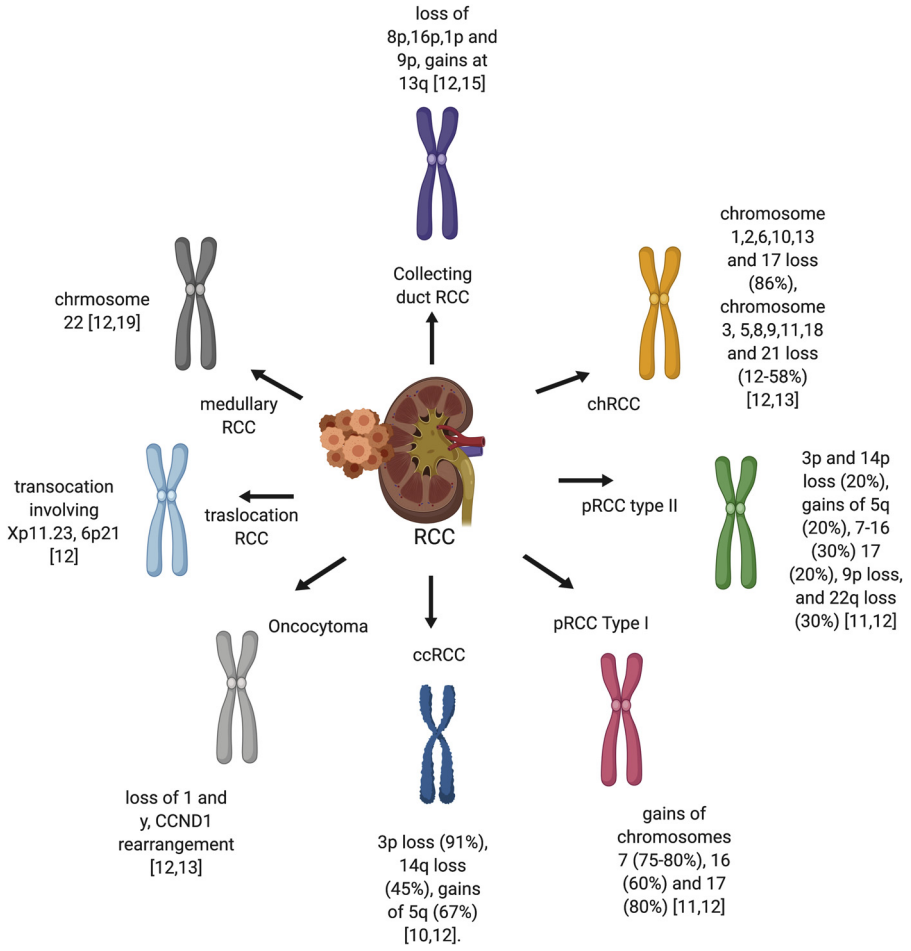

Fig. 2. Principal chromosomal rearrangements and percentage of occurrence in each tumor subtypes: clear cell carcinoma (ccRCC), papillary renal cell carcinoma type I (pRCC I) and type II (pRCC II), chromophobe renal cell carcinoma (chRCC), translocation carcinoma, collecting duct RCC, medullary RCC, oncocytoma (created by biorender.com).

From a disease orphan of a known genomic profile, we have thus moved to a spectrum of different tumors characterized by specific mutations (Fig. 1). These important genomic findings could add important information in terms of prognosis and/or response/resistance to treatment. However, due to the incredible amount of available data, a fundamental step to help us in this transition is the simplification of the current knowledge.

We shouldn't forget that tumor evolution is a dynamic process which occurs in parallel with external and physiological pressures. Despite driver or trunk mutations represent the first step in the tumor development, further mutations strongly depend also from external pressure which made a selection of different tumor cells subclones. If we consider together these ''evolutionary pressure'' steps, genomic mutation pathways of different tumors seem to convey in specific profiles.

Thus, we recognized four specific and different key external pressure factors: metabolic-, genomic-, immune- and treatment-related pressures, which could be the force moving the mutations development in each RCC tumors.

3. RCC GENOMIC STRATEGIES TO ESCAPE FROM METABOLIC PRESSURE

Mutations resulting in tumor cell metabolic alterations are probably the more common event in each RCC subtypes. It is important to observe that several different mutations inevitably lead to metabolic shift which often (but not always) results in a ''Warburg effect'' (Warburg, 1956; Vander Heiden et al., 2009a). The anaerobic degradation of glucose more than mitochondrial mediated oxidative phosphorylation is the main consequence of this shift which also leads to augmented dependence of pentose phosphate shunt, higher fatty acids production, higher intracellular level of lactate and reduction of Krebs cycle activity.

It is interesting to observe that metabolic adjustments could differ between tumor types and have different impacts on patient's survival. Indeed, expression of Krebs cycle and electron transport chain genes are lower in ccRCC and CIMP-RCC (a specific pRCC subtype characterized by a CpG island methylator phenotype), intermediate in type I and II pRCC, and higher in chRCC. On the other hand, the negative regulator of fatty acids synthesis expression of 5′ AMP-activated protein kinase (AMPK) expression was significant increased in chRCC and resulted in higher mitochondria activity. The same AMPK expression was lower in CIMP-RCC, as well as late-stage ccRCC, and resulted in poorer survival. Increased ribose sugar metabolism gene expression was observed in type 2 pRCC, CIMP-RCC, and high-stages ccRCC and was also associated with a poorer survival (Ricketts et al., 2018).

Increasing evidences seems to show an important role of mitochondrial DNA on metabolism adjustment. Oncocytoma could be divided in two main types according to different chromosomal and genetic rearrangements: Type 1 is diploid with *CCND1* rearrangements, whereas type 2 is aneuploid with recurrent loss of chromosome 1, X or Y, and/or 14 and 21, which may proceed to more aggressive eosinophilic chRCC. Notably, both type I and II oncocytomas present a higher number of mitochondria with defective activities. Although suggestive,

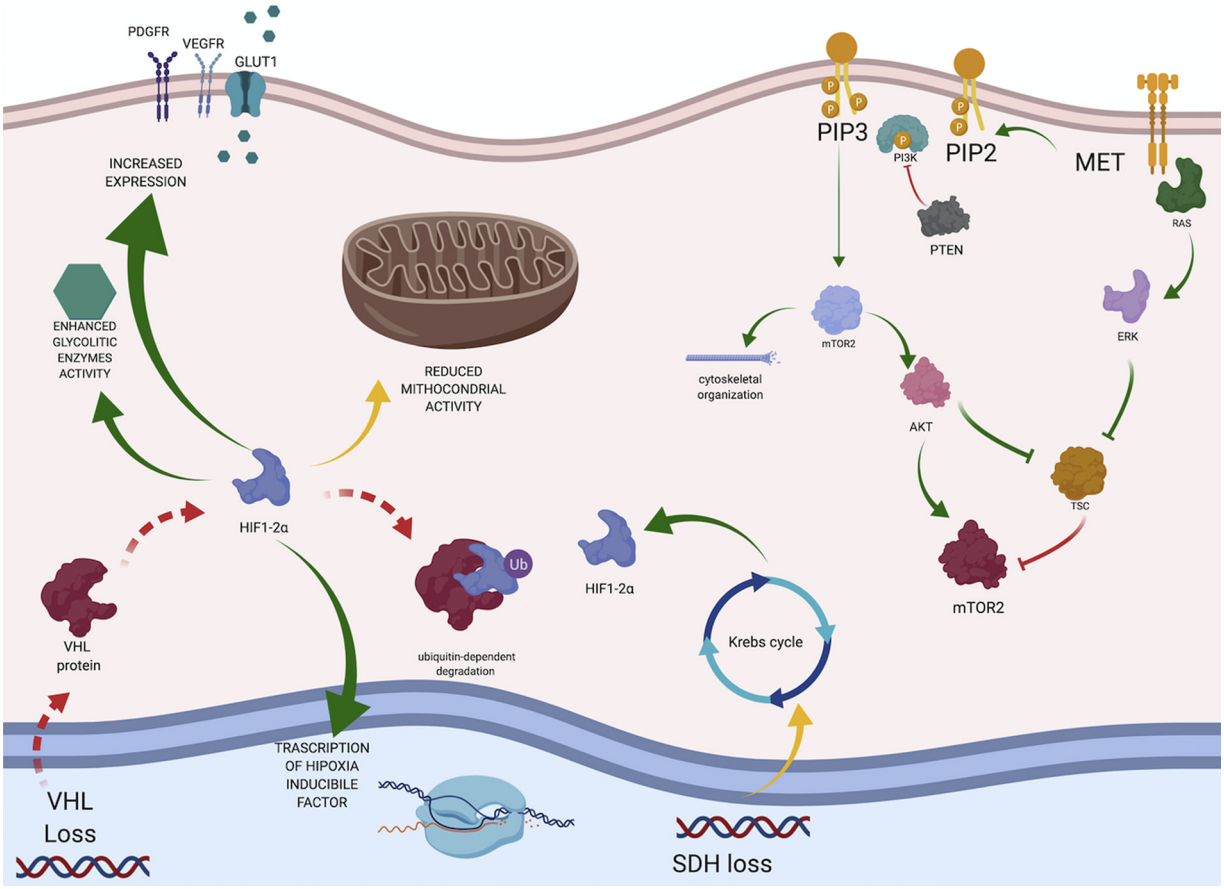

Fig. 3. Principal genomic alterations and altered pathways are summarized in Fig. 3. The *VHL* gene encodes a protein whose main task is to mediate ubiquitin-dependent degradation of the hypoxia inducible factor 1 and 2 alpha (*HIF1-2α)* (Maxwell et al., 1999). Resulting accumulation of *HIF1-2α* during hypoxia (i.e. in physiological condition) or in case of *VHL* loss (pathological condition) leads to up-regulation of hypoxia-response elements such as *VEGF, PDGF, EGF* and *GLUT1* (the glucose transporter). Moreover, *HIF1α* enhances glycolytic enzyme expression and reduces mitochondrial pyruvate consumption (Kondo et al., 2002; Wang et al., 2005). Deficit in succinate dehydrogenase leads to an impaired Krebs cycle activity and *HIF* accumulation as a response to this damage (Hsieh et al., 2017a); The *MET*-mediated activation of the phosphatidylinositol 3-kinase (*PI3K)/Akt* signaling pathway leads to augmented uptake of glucose, amino acid, enhanced glycolysis, and lipogenesis. Moreover, *MET* stimulated *RAS–Erk1/2– p90RSK* pathway, leading to an increased phosphorylation on Ser428 *of STK11* (the upstream kinase of *AMPK*), involving *MET* in the *LKB1-AMPK-mTOR* nutrient and energy sensing pathway (Gill et al., 2014; Gherardi et al., 2012; Sulpice et al., 2009). The phosphoinositide 3-kinase *(PI3K)-AKT* mammailian target of rapamycin (mTor) is a serine/threonine kinase which exists in two different complex: *mTOR* Complex 1 (*mTORC1*) and *mTORC2* (Choueiri et al., 2015; Dennis et al., 2001; Laplante and Sabatini, 2012; Wullschleger et al., 2006; Kim and Chen, 2004; Um et al., 2004; Düvel et al., 2010; Huffman et al., 2002). The first is rapamycin-sensitive, and is able to phosphorylate *S6K1* and *4E-BPs*, while the second is rapamycin-insensitive and activates several protein kinases such as *Akt* (Choueiri et al., 2015). *mTORC1* stimulate protein synthesis and induces expression of several glycolytic genes through the activation of *HIF1α* (Laplante and Sabatini, 2012Wullschleger et al., 2006). *mTORC2* regulates cytoskeletal organization and promotes cell-survival and metabolism (Choueiri et al., 2015; Dennis et al., 2001) (created by biorender.com).

it is difficult to understand if this impaired mitochondrial DNA is a consequence of metabolic genomic rearrangements, or if it is just one of the first steps in oncocytoma development; however, the identification of mitochondrial deficit and DNA impairment open a new scenario in the research of correlation between RCC development and progression (Joshi et al., 2015).

To summarize, metabolic mutations occurs quite frequently in RCC, and are associated to different survival (Fig. 3). In particular several genes may influence metabolic rearrangements in RCC:

3.1. VHL

Von Hipple Lindau is for sure the more frequent altered gene in ccRCC. Haploinsufficiency of VHL by loss of chromosome 3p in observed over 90% of tumors and occurs in childhood or late adolescence. The second copy of *VHL* is lost later during life by non-synonymous mutation or epigenetic down-regulation (Mitchell et al., 2018). Loss of *VHL* is not sufficient to induce the development of ccRCC and thus this tumor suppressor gene seems to not be so strong (in terms of genomic mutation), although it is conceivably the primary event in RCC genesis (Kapitsinou and Haase, 2008; Wei and Hsieh, 2015b). The *VHL* gene encodes a protein which interacts with the transcription elongation factors elongin C and B (*TCEB1, TCEB2*) and with several enzymatic proteins. The main task of *VHL* protein is to mediate ubiquitin-dependent degradation of the hypoxia inducible factor 1 and 2 alpha (*HIF1-2α)* (Maxwell et al., 1999). Resulting accumulation of *HIF1-2α* during hypoxia (i.e. in physiological condition) or in case of *VHL* loss (pathological condition) leads to up-regulation of hypoxia-response elements such as *VEGF, PDGF, EGF* and *GLUT1* (the glucose transporter). Moreover, *HIF1α* enhances glycolytic enzyme expression and reduces mitochondrial pyruvate consumption (Kondo et al., 2002; Wang et al., 2005).

Targeting angiogenesis has led to impressive results in our recent clinical practice, starting a still ongoing revolution(even in the present immunotherapy era) in the modern management of patients with advanced RCC (Papandreou et al., 2006; Escudier et al., 2010; Motzer et al., 2007; Escudier et al., 2007; Sternberg et al., 2010; Motzer et al., 2013a, b) (Fig. 4).

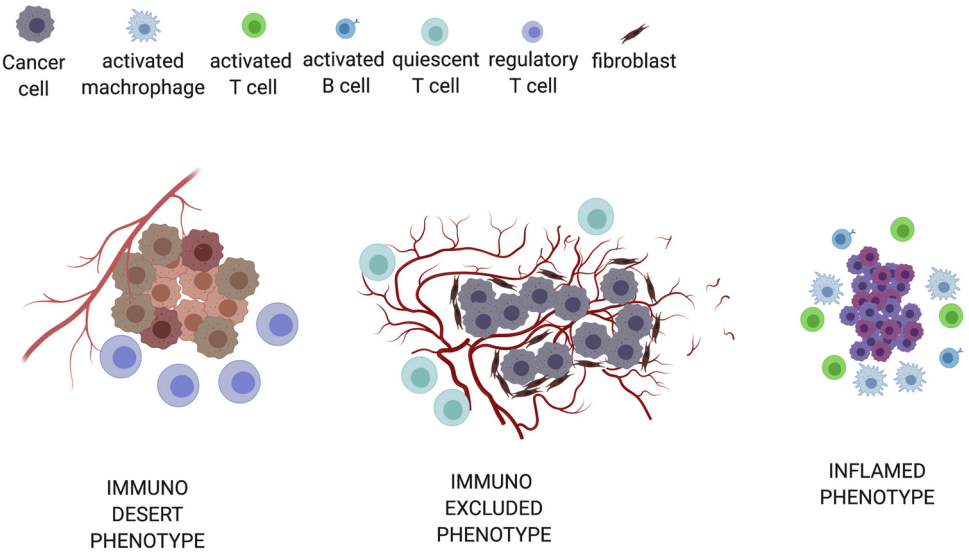

Fig. 4. In this figure are schematized possible immune-phenotype available in RCC (created by biorender.com).

Notably, an hereditary condition which results in higher *HIF* intracellular level is related to *SDH* loss:

3.2. SDH

deficit in succinate dehydrogenase subunits B, C and D results in predisposition to develop, beyond RCC, also paraganglioma, carotid body tumors, pheochromocytoma and gastrointestinal stromal tumors. Loss of *SDH*, a key enzyme which explicate its activity on Krebs cycle electron transport chain, leads to an impaired Krebs cycle activity and *HIF* accumulation as a response to this damage (Hsieh et al., 2017a); it is observed in a very limited number of RCC characterized by male predominance, early age of presentation, unwillingness to metastasize, and negative immunostaining for *SDHB* (Santoni et al., 2018a).

3.3. MET

MET (Mesenchymal Epithelial Transition or Hepatocyte Growth Factor Receptor) is the tyrosine kinase receptor of Hepatocyte Growth Factor/Scatter Factor (HGF/SF). This gene seems to assume shared signaling intermediates with *VEGFR*. In particular, *ERK, MAPK, AKT* and *FAK* could be activated by both these receptors. *MET* activation can also induce *VEGF*A expression and angiogenesis through *SHCs* (*SRC* homology 2 domain containing proteins) (Gill et al., 2014; Gherardi et al., 2012; Sulpice et al., 2009). In addition, angiogenesis could be supported through an inhibition of *HGF/SF* mediated by thrombospondin 1 (*TSP1*), which is a negative regulator of angiogenesis. Furthermore, *HIF1α* and *HIF1β* hyper-expression lead to *MET* expression. The *MET*-mediated activation of the phosphatidylinositol 3-kinase (*PI3K)/Akt* signaling pathway leads to augmented uptake of glucose, amino acid, enhanced glycolysis, and lipogenesis. Moreover, *MET* stimulated *RAS–Erk1/2– p90RSK* pathway, leading to an increased phosphorylation on Ser428 *of STK11* (the upstream kinase of *AMPK*), involving *MET* in the *LKB1-AMPK-mTOR* nutrient and energy sensing pathway (Gill et al., 2014; Gherardi et al., 2012; Sulpice et al., 2009).

Several drugs targeting this pathways are under investigation (NCT02019693, NCT02761057, NCT03091192, NCT01835158, NCT03428217, NCT03170960). Of interest cabozantinib is a multitarget inhibitors which acts mainly on angiogenesis and also on other pathways including MET. This drug has become a new standard in management of metastatic ccRCC (Zhang and Su, 2003).

3.4. mTOR

The phosphoinositide 3-kinase *(PI3K)-AKT* mammailian target of rapamycin (mTor) is a serine/threonine kinase which exists in two different complex: *mTOR* Complex 1 (*mTORC1*) and *mTORC2* (Choueiri et al., 2015; Dennis et al., 2001; Laplante and Sabatini, 2012; Wullschleger et al., 2006; Kim and Chen, 2004; Um et al., 2004; Düvel et al., 2010; Huffman et al., 2002). The first is rapamycin-sensitive, and is able to phosphorylate *S6K1* and *4E-BPs*, while the second is rapamycin-insensitive and activates several protein kinases such as *Akt* (Choueiri et al., 2015).

mTORC1 stimulate protein synthesis and induces expression of several glycolytic genes through the activation of *HIF1α* (Laplante and Sabatini, 2012; Wullschleger et al., 2006).

mTORC2 regulates cytoskeletal organization and promotes cell-survival and metabolism (Choueiri et al., 2015; Dennis et al., 2001). Notably, *mTOR* acts as a nutrient-sensing pathway maintaining homeostasis through the regulation of protein synthesis and amino-acids availability. On the other hand, *mTORC1* controls also adipogenesis through the *S6K1* mediated expression of *PPARγ,* stimulating also fatty acids and cholesterol synthesis regulating the activity of the transcription factor sterol regulatory element-binding protein-1c *(SREBP-1c)*. *PPARγ* adipogenic activity is also promoted by *mTORC1* promoted lipin 1 (*Lpin1*) phosphorylation which leads to increased triglyceride synthesis. It is intriguing to highlight that *mTORC1* is also associated to increased insulin secretion, insulin resistance in adipose tissue, increased hepatic gluconeogenesis activity, *HIF1α* levels and hepatic ketogenesis inhibition (Laplante and Sabatini, 2012; Wullschleger et al., 2006; Kim and Chen, 2004; Um et al., 2004; Düvel et al., 2010; Huffman et al., 2002).

According to the branched evolution model, *mTOR* pathway's mutations occurs in a second time after the development of ''trunk'' mutations in RCC (Sanfrancesco and Cheng, 2017). Thus, the high frequency of *mTOR* alterations in RCC could explain the positive results achieved with selective inhibitor targeting this altered pathway (Peterson et al., 2011; Motzer et al., 2008; Hudes et al., 2007; Di Nunno et al., 2018a).

Notably, there are several hereditary condition as well as different genes which mutation results in mTOR upregulation. These genes are:

3.4.1. TSC1/TSC2

Tuberous Sclerosis is an autosomal dominant disorder caused by Tuberous Sclerosis Complex 1 or 2 (*TSC1/2*) germline mutations. This

condition usually leads to the development of different renal tumors including angiomyolipoma, ccRCC and pRCC, as well as to the development of astrocytomas, facial angiofibromas, ungueal fibromas, and cardiac rhabdomyomas (Hsieh et al., 2017a). These two genes encoded hamartin and tuberin (respectively encoded by *TSC1 and 2*), which mainly act as GTPase protein inhibiting Ras homolog enriched in brain (*Rheb)*. *Rheb* inhibition leads to a missed activation of *mTORC1* kinase activity. Thus, the *TSC1/TSC2* loss leads to *mTOR* pathways up-regulation with the already mentioned effects on cell metabolism (Di Nunno et al., 2017; Crino et al., 2006; Guo et al., 2014).

3.4.2. Folliculin

the tumor suppressor gene folliculin interacts with *FNIP1* and *FNIP2* to develop an energy sensor complex which is able to cooperate with *AMPK* and reduce *mTOR* activity (Baba et al., 2006; Hasumi et al., 2008). Loss of folliculin is an autosomal dominant syndrome also known as Birt-Hogg-Dubè syndrome, which clinically results in fibrofolliculomas, pulmonary cysts, pneumothorax, and development of oncocytoma, as well as of chRCC (Hsieh et al., 2017a).

3.5. BAP1

BRCA1 associated protein (*BAP1*), with *PBRM1* and *SETD2*, is one of the most frequently altered chromatin remodeling/histone methylation pathways in RCC (Cancer Genome Atlas Research Network, 2013, 2016; Ricketts et al., 2018). Even if *BAP1* acts as an oncosuppresor gene, and studies carried out on this gene also suggest an important role on DNA damage repair, further evidences seems to correlate this gene to several metabolic functions. *BAP1* and *PBRM1* were recognized as mutually exclusive mutations and combined *BAP1* and *PBRM1* loss happens in very few case, ultimately resulting in a rhabdoid tumoral phenotype with aggressive clinical behavior (Peña-Llopis et al., 2012). Regarding the prognostic function of *BAP1* in RCC, loss of these two genes do not seems to result in a tumor development advantage (Peña-Llopis et al., 2012); however, several evidences suggest that tumors arising *BAP1* loss were associated to a worst prognosis.

A study carried out on 16 individuals with heterozygous germline *BAP1* mutations (*BAP1 +/-)* showed an increased aerobic glycolysis and impaired mitochondrial respiration. Fibroblasts cultures obtained from plasma samples showed a higher glucose consume and lactate production (Bononi et al., 2017a). In cytoplasm, Carbone and Colleagues revealed a substantial cytosolic activity of *BAP1* in promoting apoptosis by stabilizing type 3 inositol-1,4,5-trisphosphate receptor (*IP3R3*) and consequently modulating calcium ($Ca2+$) release from the endoplasmic reticulum (Bononi et al., 2017b; Vander Heiden et al., 2009b).

Recently, Zhang et al proposed an interesting mechanism by which *BAP1* regulates ferroptosis. In this study the Authors carried out an unbiased genome-wide analysis on *UMRC6* cells (*BAP1* deficient renal cell cancer line). In brief, according to their analyses, *BAP1* inactivation leads to an up-regulation of the Solute Carrier family 7 member 11 (*SLC7A11*). This last protein plays a key role on the intracellular cystine transfer. Cystine depletion results in ferroptosis, which is a form of regulated cancer death mainly due to the oxidative cellular stress resulted from the overproduction of reactive oxygen species (*ROS*).

Loss of BAP1 leads to a missed H2Aub deubiquitination of *SLC7A11*, resulting in enhanced *SLC7A11* activity and finally in higher intracellular cystine levels. Cysteine balances redox stress preventing ferroptosis (Zhang et al., 2018).

As *BAP1* loss seems to confer sensitivity to poly *ADP* ribose polymerase (*PARP*) inhibitors several of these agents are under investigation alone or with other target agents (as an example, agents targeting *AKT*, with NCT03207347 as the only active trial in RCC).

Finally, the accumulation of ubiquitinated histone H2A resulting from *BAP1* loss provides also a strong rational to evaluate the role of hystone deacetylase inhibitors which are currently under investigation (NCT03592472, NCT03552380, NCT02619253, NCT03501381, NCT03024437, NCT02890069).

3.6. FH

Although loss of fumarate hydratase function is an infrequent events leading to hereditary leiomyomatosis and renal cell carcinoma (HLRCC) cancer syndrome, the metabolic alteration resulting from this disease appears of particular interest (Hsieh et al., 2017a).

The *FH* enzyme catalyzes the conversion of fumarate to malate in mitochondrial matrix. Furthermore, it has an important role on aminoacid metabolism. With the loss of *FH* activity, the cell is no more able to use oxidative phosphorylation as the main source of ATP production. Increasing intra-cellular levels of fumarate leads also to *HIF1α* stabilization and up-regulation in a *VHL* independent manner. Also reactive oxygen species *(ROS)* resulting from anaerobic glucose consumption leads to *HIF1α* stabilization. Cell prevents ROS mediated damage through the activation of an antioxidant response, which results from a degradation of Nuclear factor (erythroid derived 2)-like 2 (*NrF2)* mediated by *KEAP1* (Kelch-like ECH-associated protein 1). The metabolic shift resulting by these mechanisms lead to the development of type II pRCC characterized by high Fuhrman grade, large eosinophilic nucleoli with aggressive clinical behaviors, and poor prognosis (Massari et al., 2015). Drugs targeting angiogenesis, lactate dehydrogenase-A (enzyme promoting fermentative glycolysis), or drugs able to increase ROS levels through proteasome inhibition resulting in tumor cell apoptosis (e.g. bortezomib) are under investigation, although preliminary results do not seem exciting (Kondagunta et al., 2004; Davis et al., 2004).

In conclusion, it is important to consider that the majority of the genes included in this paragraph play an important role also in other pathways different from metabolic rearrangement. Indeed, as we will see in next paragraph some of these genes play a key role in tumor escape from other external pressure. Nonetheless, it seems that metabolic alterations occur in a transversal way, suggesting the achievement of metabolic advantages is a key step for RCC resulting in tumor development and progression. Future studies will help us to elucidate if other genes known to be associated to other pathways could play an indirect contribute on metabolism rearrangement in RCC.

4. Escape from genomic control pressure achieving a tumor survival benefit

A not negligible percentage of altered genes in RCC have, as their major task, the maintenance of cellular homeostasis by regulation of cell growth and division in order to prevent excessive cellular proliferation and leading to programmed cell death in case of DNA damages.

SETD2, PBMR1, BAP1, KDM5C, PTEN, P53 and *CDKN2A* are frequent altered genes across all RCC subtypes (Ricketts et al., 2018). Mutated genes shared by ccRCC, pRCC and chRCC are *TP53* and *PTEN*; however, the prognostic impact of these genes assume a different role in each subtypes. Indeed, *TP53* has been related to poor prognosis only in ccRCC and pRCC while *PTEN* resulted in worst overall survival only in ChRCC. *PBMR1, SETD2* and *BAP1* mutations occurs in both ccRCC and pRCC while these have not been observed in chRCC. Of note, *PBRM1* alterations have been related to poor prognosis only in papillary type I RCC while no correlation with survival has been observed in ccRCC.

Mutation, hypermethylation or deletion of *CDKN2A* can be detected in all tumor subtypes and in the 100% of CIMP-PRCC. The loss of *CDKN2A* leads to poor prognosis in all tumor subtypes (Ricketts et al., 2018).

Again, the frequent observation of mutations across gene regulating cell cycle reveals that one of the most important gain achieved by tumor cells could be identified in the escape between the mechanisms controlling tumor division and cell growth. In this line is important to

Table 1
This table summarized principal genes involved in genomic escape.

Gene	function
SETD2 (Piva et al., 2015a, b; Carvalho et al., 2014)	*SETD2* loss are associated with loss of DNA methylation on non-promoter regions. The resulting methylation leads to a higher chromatin accessibility and genomic transcription.
PBRM1 (Piva et al., 2015a, b; Kakarougkas et al., 2014; Brownlee et al., 2014)	it regulates histone accessibility and DNA transcription. Another role carried out by *PBRM1* involve the transcriptional silencing induced by DNA double strands damage, and the negative regulation of several genes involved in cellular proliferation and chromosomal instability
BAP1 (Peng et al., 2018; Di Nunno et al., 2019)	In the nucleus, BAP1 interacts with *ASXL1/2* to form the core Polycomb complex *PR-DUB* which induces chromatin modification associated with gene repression by deubiquitinating histone H2A at Lys119 (*H2AK119ub*) and *HCFC1*. *By deubiquitinating HCF-1, an E2F promoter co-regulator, BAP1 indirectly modulates the activity of E2F*
KDM5C (Hsieh et al., 2017a, b)	The Lysine-specific demethylase 5C in an enzyme which act mainly regulating transcription and chromatin remodeling. Of note, as observed also with *PBRM1*, mutations in *KDM5C* seem to be mutual exclusive with *BAP1* mutations
CDKN2A (Li et al., 1994; Rayess et al., 2012; Witkiewicz et al., 2011)	The cyclin-dependent kinase inhibitor 2A gene encodes two tumor suppressor protein: the *p16INK4a* and p14arf. *P14INK4A* is able to bind and inhibit the *CDK4/6* preventing its kinase activity and so preventing the phosphorylation of RB protein
TP53 (Vogelstein et al., 2000; Bousquet et al., 2015)	This protein mediates DNA repair, cell cycle arrest, and initiates apoptosis
PTEN (Chu and Tarnawski, 2004)	The phosphatase and tensin homolog *PTEN* protein acts as tumor suppressor protein. Its function is mainly explicating through the de-phosphorylation of the phosphatidylinositol (3,4,5)-triphosphate resulting in an inhibition of the *AKT* signaling pathway
NF2 (Johnson and Halder, 2014)	Mutations of *neurofibrin 2* gene resulted in an inhibition of *MERLIN* tumor suppressor protein which regulate the Hippo pathway which through negative regulation of two transcriptional co-activators: *YAP* and *TAZ* regulates cell proliferation, invasion and metastatic spread of RCC

observe which are the mechanisms by which the loss of these genes leads to tumor development and progression (Table 1).

4.1. SETD2, PBMR1 and BAP1

SETD2, PBMR1 and BAP1 genes are all located on chromosome 3. Thus, as previously written, trunk mutation of chromosome 3p is one of the earlier events on RCC tumor development, and due to the nearest of these gene to other key sequences (*VHL*) is easy to understand why these genes are often mutated, especially in ccRCC. These genes play also an important role in chromatin status regulation (Piva et al., 2015a, b).

4.2. SETD2

SET Domain containing 2 is an enzyme which acts as histone methyltransferase specific for lysine 36 of histone H3. *SETD2* loss are associated with loss of DNA methylation on non-promoter regions. The resulting methylation leads to a higher chromatin accessibility and genomic transcription. This gene plays also a key role on DNA damage repair as the lysine 36 trimethylation of histone H3 cooperate with *ATM* and *p-54* on recombinant repair of double strands DNA damages (Piva et al., 2015a, b; Carvalho et al., 2014).

4.3. PBRM1

Polybromo1 product is a subunit of the *SWI/SNF* transcription-modulating chromatin remodeling complex. Through its six bromodomains, *PBRM1* binds acetylated residues on histones with different target affinity showing a specific histone affinity. By this binding, it regulates histone accessibility and DNA transcription. Another role carried out by *PBRM1* involve the transcriptional silencing induced by DNA double strands damage, and the negative regulation of several genes involved in cellular proliferation and chromosomal instability (Piva et al., 2015a, b; Kakarougkas et al., 2014; Brownlee et al., 2014).

4.4. BAP1

As previously observed, *BAP1* plays an important role in metabolic regulation. In the nucleus, BAP1 interacts with *ASXL1/2* to form the core Polycomb complex *PR-DUB* which induces chromatin modification associated with gene repression by deubiquitinating histone H2A at Lys119 (*H2AK119ub*) and *HCFC1*. Peng H. et al recently demonstrated how the interaction between *BAP1* and ASXL2 is direct, and that this binding stimulates BAP1 hydrolase activity. Mutations in *BAP1-ULD*, which occurs in several tumors, abolish the interaction with *ASXL2* resulting in a missed stimulation on *BAP1* activity. By deubiquitinating *HCF-1*, an *E2F* promoter co-regulator, *BAP1* indirectly modulates the activity of *E2F* (Peng et al., 2018; Di Nunno et al., 2019).

4.5. KDM5C

The higher incidence of ccRCC in male patients may partially be accounted by monoallelic inactivation of the chromatin remodeling gene *KDM5C* on the X chromosome (Hsieh et al., 2017a). The Lysine-specific demethylase 5C in an enzyme which act mainly regulating transcription and chromatin remodeling. Of note, as observed also with *PBRM1*, mutations in *KDM5C* seem to be mutual exclusive with *BAP1* mutations. Mutations in this gene have also been related to long response to sunitinib (Hsieh et al., 2017b).

4.6. CDKN2A

The cyclin-dependent kinase inhibitor 2A gene encodes two tumor suppressor protein: the *p16INK4a* and p14arf. *P14INK4A* is able to bind and inhibit the *CDK4/6* preventing its kinase activity and so preventing the phosphorylation of RB protein. The missed phosphorylation of RB prevents RB inactivation and blocks *E2F1* target genes transcription which is essential for G1/S transition. The *p16INK4a/RB* pathway is also able to activate protein kinase C delta by the induction of reactive oxygen species resulting in an irreversible cell cycle arrest (Li et al., 1994; Rayess et al., 2012; Witkiewicz et al., 2011).

P14ARF is able to inhibit *MDM2*, which is an *E3* ubiquitin ligase protein controlling and preventing *TP53* activity. Loss of *P14ARF* leads to *TP53* dysfunction resulting in missed G2 phase cycle arrest.

On the other hand, *P14ARF* is also able to interact with *RB* through *E2F* preventing G1 to S cell cycle progression (Eymin et al., 2003 Mar; Rizos et al., 2007).

4.7. TP53

the *TP53* protein encoded by *p53* gene is a key tumor suppressor protein which is inactivated in different tumors. As already known, this protein mediates DNA repair, cell cycle arrest, and initiates apoptosis

(Vogelstein et al., 2000). Curiously, Bousquet et al. performed compared *TP53* expression between primary tumor and metastases samples in eight RCC patients. They were able to identify *TP53* mutated clones only in few tumor cells of primary tumors samples, while the same TP53 mutated clones were significantly increased in metastatic sites. These findings reinforced the evidences suggesting that a subclonal evolution leads to the development of different tumor cell subclones which presented a different metastatic predisposition. In this optic, the achievement of TP53 loss seems to be a key step for the development of metastasis (Bousquet et al., 2015).

4.8. PTEN

The phosphatase and tensin homolog *PTEN* protein acts as tumor suppressor protein. Its function is mainly explicating through the dephosphorylation of the phosphatidylinositol (3,4,5)-triphosphate resulting in an inhibition of the *AKT* signaling pathway (Chu and Tarnawski, 2004).

4.9. NF2

Mutations of *neurofibrin 2* gene resulted in an inhibition of *MERLIN* tumor suppressor protein which regulate the Hippo pathway which through negative regulation of two transcriptional co-activators: *YAP* and *TAZ* regulates cell proliferation, invasion and metastatic spread of RCC (Johnson and Halder, 2014).

Despite a great number of potential emerging targets, no specific drugs are under investigation with the exception of Niraparib (a *PARP* inhibitor) in solid tumors harboring *BAP1* or other DNA Damage Response deficit (NCT03207347). This reflects the difficult replacement of a tumor suppressor protein loss. However, in past few years a new class of agents known as oncolytic viruses is gaining increasing interest. Indeed. recombinant virus able to exhibit a specific tropism and able to express specific proteins which are loss in tumor cells represents an interesting and suggestive approach which is currently under investigation (Delwar et al., 2016; Liu et al., 2018; Mollica et al., 2019a).

5. ESCAPE FROM IMMUNE-PRESSURE

As known, before the advent of tyrosine kinases inhibitors able to target specific altered pathways of the disease, old immunotherapeutic agents such as IL-2 and INFα were the only available treatments option for patients with advanced disease. In particular IL-2 was associated to clinical activity and complete response in a small but not negligible percentage of patients. In more recent years, thanks to the advent of new technologies and to a better knowledge of its molecular alterations, RCC was demonstrated to be a tumor with a mild to high percentage of somatic mutations, resulting in higher percentage of neo-antigens, and finally high immunogenic power (Lawrence et al., 2013). This observation prompted the use of a new class of drugs known as immune-checkpoint inhibitors, which have drastically changed the course of the disease. These agents have been developed to solve a well known and important mechanism of immune escape, thanks to which cancer cells inhibit T-cells immune-response: the interaction between Programmed Death Receptor Ligands 1 and 2 (PD-L1/2) and the programmed death receptor 1 (PD-1) expressed from T-cells. Through this interaction immune response does remains in a quiescent status even if tumour express a high percentage of immune-responsive neo-antigens. Thus, the anti PD-1 agent Nivolumab and more recently the combination between Nivolumab and Ipilimumab (an antibody targeting the Cytotoxic T-Lymphocyte antigen 4) have shown to significantly increase survival and improve other clinical outcomes in previously treated patients (Nivolumab) and in moderate and high risk (according to IMDC criteria) previously untreated patients (Nivolumab-Ipilimumab) with advanced ccRCC (Motzer et al., 2015a, 2018).

However, despite PD-L1 expression by tumor cells should be considered as a key adaptive mechanism which make them able to escape from immune recognition, several other strategies allow them to achieve the same result. An interesting review carried out by Chen et al. summarize the main tumor immune-phenotypes and so, tumor cells could present (Chen and Mellman, 2017):

5.1. Immune desert tumor phenotype

Tumors without immune infiltration mainly due to the missed expression of neo-antigens, the inhibited immune-cells recruitment due to the activation of Regulatory T cells and/or the secretion of suppressive cytokines or the absence of co-stimulator or primers able to start immune-response.

5.2. Immune excluded tumors

Immune excluded tumors are tumors which adopts several strategies, including the development of angiogenesis and extracellular matrix, to hide from immune-cells

5.3. Inflamed tumors

Inflamed tumors are tumors associated to a high immune infiltrate in which immune response is inhibited from different strategies such as the expression of PD-L1/2.

Of course, these are only some of the strategies adopted by tumor cells to escape from immune system.

As observed in other tumors, to date there is no single gene which has been shown to be dedicated to immune pressure escape; thus, it is probable that different mutated genes may cooperate one with the other to allow this evolution gain. Interestingly, alteration in tumor suppressor genes or in genes able to promote DNA repair does not seem to be ideal candidates foe this role. Promotion in DNA transcription resulting from their loss, as well as the transcription of altered DNA, could result in higher expression of abnormal proteins, and possibly also in higher concentration of neo-antigens. However, what is known is that ccRCC, compared to pRCC and chRCC, express an higher expression of specific genes such as *PDCD1* and *CD247* (expressing PD1 and PD-L1) (Ricketts et al., 2018; Chen et al., 2016).

Moreover, the Cancer Genome Atlas Comprehensive Molecular Characterization of RCC performed an extensive immune signature gene analysis demonstrating that, with the exception of the Th17 (more expressed in ChRCC), IL-8 and $CD56^{bright}$NK (more expressed in pRCC) cell genes, the others were significantly upregulated in ccRCC compared to pRCC and chRCC (Ricketts et al., 2018). Notably, CIMP-RCC tumors presented a more ccRCC immune-gene signature. Authors highlighted also that the Th2 gene signature (known to be associated to T-Cell regulatory activity) was significantly up-regulated in a high percentage of ccRCC, in all CIMP-pRCC and in some pRCC and chRCC and was associated to poor prognosis regardless tumor hystotypes (Ricketts et al., 2018).

Despite these evidences, it has been demonstrated that tumor mutational burden is a key factor related to immune-checkpoint inhibitors response and clinical outcomes, as it strictly correlates with neo-antigen productions (Rizvi et al., 2015; Yarchoan et al., 2017). This axiom seems to fit also for ccRCC. Indeed, results of checkmate214 trial clearly showed that immunotherapy acts better on patients with intermediate-high risk according to IMDC, and thus in patients with more clinical aggressive tumors. However, the supposed association: ''the higher mutation burdens, the higher prognostic risk'' has not been confirmed (de Velasco et al., 2016). Moreover, also sarcomatoid component of ccRCC does not seem to be associated to higher mutational burden, and so this variable does not seem associated to worst clinical or histological features of the disease (Malouf et al., 2016).

Despite no singular gene has been strictly associated to tumor immune escape, it is sure that this is a key strategies adopted by a not

negligible percentage of tumors. Future studies should be aimed to understand which genes are controlling more than other these important strategies; along this line, studies exploring gene panels or immune specific signatures of different tumor samples appears of particular interest. However, we shouldn't forget that immune-response is a dynamic process and so a ''snapshot'' and evaluation of a single samples could hide a large amount of information. What we do presently know is that immune-checkpoint are a new treatment paradigm in the management of advanced ccRCC. Current trends in immune-checkpoint inhibitors trials are leading to explore these agents in combination with other different target inhibitors (Santoni et al., 2018b; Massari et al., 2017, 2019; Ciccarese et al., 2017; Di Nunno et al., 2018b). Very recently new trials showed impressive results on first line setting.

In KEYNOTE-426 861 patients were randomized to receive the combination between pembrolizumab and axitinib or sunitinib (Rini et al., 2019a). Co-primary end points were OS and PFS in all patients treated (regardless IMDC risk score) while ORR, duration of response and safety were secondary end points. Patients randomized in combination arm showed a significantly better OS and PFS compared to sunitinib arm. Also ORR (59.3% vs 35.7%) and duration of response (not reached vs 15.2 months) favored the combination arm.

The combination of avelumab and axitinib has been assessed in JAVELIN Renal 101 trial (Motzer et al., 2019). In this study 886 patients were randomized to receive the combination or sunitinib. Co-primary end points were PFS and OS among patients with PD-L1 positive tumors (assessed by Ventana SP263 assay). OS and PFS among all population in study as well as ORR, safety and patients reported outcomes were secondary end points. Improved PFS was observed in both PD-L1 positive and overall population. OS analysis failed to demonstrate a significant benefit for combination arm, however this may reflect a still immature follow up. ORR was significantly improved in combination arm in both PD-L1 positive (55.2% vs 25.5%) and overall population (51.4% vs 25.7%).

The last published trial was IMmotion151 (Rini et al., 2019b), which compared the combination between atezolizumab and bevacizumab over sunitinib. Overall, 915 patients were randomized to receive the combination or sunitinib. Co-primary end points were: PFS in patients with PD-L1 positive disease and OS in intention-to-treat population. OS in PD-L1 positive patients, PFS in intention-to treat population, ORR and safety were secondary end points. Combination showed to improve PFS in PD-L1 positive patients and intention-to treat population however OS in PD-L1 expressing patients and intention-to treat population was similar between the two arms. Patients in combination arm achieved an ORR of 43% (PD-L1 +) and 37% (overall) while patients treated with sunitinib obtained an ORR of 35% (PD-L1 +) and 33% (overall).

6. TUMOR ESCAPE FROM TREATMENT PRESSURE

Apart from other "usual" factors of pressure that RCC cancer cells have to overtake, another important external pressure to consider is due by the treatments adopted in our clinical practice to counteract its presence. Indeed, our treatments directly and indirectly modulate genetic expression of RCC inducing an ''evolutionary pressure'' by selecting only sub-clones able to survive regardless systemic treatment (Mollica et al., 2019b). In this optic, the identification of genes related to tumor treatment resistance appears of particular interest for the development of new target agents.

6.1. Resistance to standard VEGF/VEGFR inhibition

Several supposed mechanisms of resistance to antiangiogenic treatments exist (Itatani et al., 2018). As far as RCC, there are some particular genes which appears of particular interest. As already described, mTOR mutations could result in several metabolic rearrangements, but also in a *HIF1α* accumulation (Choueiri et al., 2015; Dennis et al., 2001; Laplante and Sabatini, 2012; Wullschleger et al., 2006; Kim and Chen, 2004; Um et al., 2004; Düvel et al., 2010; Huffman et al., 2002). *MET* could also be a key gene associated to resistance to *VEGFR* inhibition, as it represents another pathway able to stress angiogenesis pathways (Gill et al., 2014; Gherardi et al., 2012; Sulpice et al., 2009). As a consequence, several drugs targeting these up-regulated pathways have been evaluated among clinical trials, with excellent results (Zhang and Su, 2003; Peterson et al., 2011; Motzer et al., 2008; Hudes et al., 2007; Di Nunno et al., 2018a). Other interesting target under investigation are represented by:

6.1.1. Fibroblast Growth Factor receptor (FGFR)

Through the *FGF/FGFR* pathway, essential during embryogenic development, several downstream pathways (*MAPK/ERK, PI3K/AkT, STAT*, diacylglycerol protein kinase C and inositol triphosphate) are activated. Despite alterations of this gene have been found in a very few percentages of RCC specimens, probably it plays a key role to the development of sunitinib resistance (Tsimafeyeu et al., 2011; Tran et al., 2016). Several FGFR inhibitors have been tested with conflicting results (Motzer et al., 2014; Schmidinger, 2014); however, more recently the association between lenvatininb (a multikinase inhibitor able to inhibit also *FGFR*) and everolimus demonstrated a very interesting activity in previously treated patients with RCC (Jonker et al., 2011). Of interest, this is one of the first cases of multi-target inhibition strategies applied on clinical practice; thus, the blockade of other possible treatment escape pathways represents one interesting approach which is already under investigation in different trials (NCT03173560, NCT02811861, NCT02915783).

6.1.2. Other mechanisms involved in anti-angiogenetic treatment resistance

Correlation between the immune-system and angiogenenic pathways are of particular interest. Several studies showed that the hyperexpression of IL-8 leads to *VEGF* mRNA transcription and autocrine *VEGFR-2* activation. *IL-6* leads also to *AKT/mTOR* and *STAT3* cascade resulting in *VEGF* expression. High levels of both *IL-6* and *IL-8* have been associated to poor prognosis in RCC, and the inhibition of *IL-6* receptor seems to lead to restored anti-angiogenic activity (Eisen et al., 2012; Motzer et al., 2015b; Ishibashi et al., 2017; Martin et al., 2009). *PDGF, TFG-alpha, EGF* are other interesting pathways which could leads tumor escape from anti-angiogenesis treatment (Itatani et al., 2018; Santoni et al., 2014).

6.2. Resistance to mTOR inhibitors

There are several supposed mechanisms by which tumors may achieve an escape from mTOR pathway inhibition (Santoni et al., 2014). Since the activity of available inhibitors is explicated through the inhibition of *mTORC1* complex, *mTORC2* could supply to this inhibition leading to *AKT* activation. This is mainly due to a reduced phosphorylation (mediated by mTORC1) which leads to activation of *mTORC2*, and finally to the activation of *AKT* through *mTORC2* mediated phosphorylation. On the other hand, the lack of *mTORC1* mediated activation of the two key proteins *GRB10* and *S6K1* could lead to lack of negative feedback regulation of AKT activation and thus to an hyperactivation of this pathway (Sarbassov et al., 2006; Yu et al., 2011; Harrington et al., 2004). *PTEN* loss seems to be associated to *mTOR* inhibitors enhanced activity mainly due to the missed inhibition of *PI3K/AKT* resulting from the loss of this gene. However, other evidences seem to not correlate *PTEN* levels to *mTOR* inhibitors' outcome in patients with RCC. *PI3K/AKT* activation may result also from *ERK/MAPK* activation resulting from *mTORC1* inhibition (Neshat et al., 2001; Figlin et al., 2009). Another interesting mechanism of resistance could be the direct activation of *AKT* carried out by reactive oxygen species. Indeed, higher percentage of these species resulting from metabolic shift or other metabolic alterations may lead to direct

phosphorylation of *AKT* and *PI3K* promoting cell survival and preventing apoptosis (Santoni et al., 2014; Okoh et al., 2013). Strategies under investigation to overcome and prevent resistance to ''classic'' *mTORC1* inhibitors are investigating the role of specific inhibitors of both *mTORC1/2, PI3K* and *AKT* (NCT01480154, NCT02724020).

6.3. Resistance to immune-checkpoint inhibitors

Immune-checkpoint inhibitors have been recently included in our clinical practice, but very few data about specific genes involved in PD-1/PD-L1 and CTLA-4 inhibitor resistance are available. Nonetheless, one interesting hypothesis is that resistance to these drugs directly reflect metabolic alterations of the disease. In this line Ascireto et al. carried out a RNA expression analysis and real time PCR gene expression analysis on PDL1 + regions of 13 RCC samples (Ascierto et al., 2016). They identified a significantly increased expression of *UGT1A6* in patients not achieving a clinical benefit from immune-checkpoint inhibitors despite PD-L1 positivity. This gene seems to be associated to toxin clearance, and thus it might play a key role in the development of resistance to immune-checkpoint inhibitors. Despite this, no final conclusions could be obtained from this study; indeed, the supposed association between metabolic alterations and response/resistance to immune-checkpoint inhibitors appears of particular interest and should be better investigated in further (and larger) studies. An interesting approach for the evaluation of mechanisms related to ''new'' immunotherapy treatment tumor escape could be the evaluation of tumor associate microenvironment, which could directly reflect the immune gene signature of a specific tumor. Indeed, as already described in previous paragraphs, tumor cells could adopt several systems to escape from immune pressure. These strategies directly correlate with the composition of the microenvironment defined as the immune cells surrounding tumor tissue. Recently, an extensive immune-profiling study of 74 ccRCC samples suggested that tumor microenvironment could be a key factor able to modulate response or resistance to immune checkpoint inhibitors (Chevrier et al., 2017).

Increasing knowledge of resistance mechanisms is a key issue for the development of new treatment strategies and thus also new drugs. Moreover, the understanding of acquired or pre-treatment genes able to lead to treatment resistance is also important to drive therapeutic decisions in our clinical practice preventing also toxicities in patients unlikely to respond to a specific treatment. (Ciccarese et al., 2016a; Inno et al., 2017; Ciccarese et al., 2016b). Current trend is the co-inhibition of multi-target through the adoption of combination treatment strategies. These involve combination between immune checkpoint inhibitors and targeted therapies (NCT02811861, NCT02684006, NCT03141177, NCT024220821, NCT0285331). It is possible that the results of this study will further revolutionize our clinical practice and will give us further key informations regarding altered gene interaction in RCC.

7. Conclusion

Just few years ago we totally ignored the genomic complexity of RCC; nowadays, we have started to understand this complex landscape and to apply our findings in clinical practice. However, so far we have just begun to explore a very exciting and unknown world. The next challenge will be to better understand tumor heterogeneity. Along this line, it will be quite important to evaluate not only genomic expression of both primary and metastatic tumor samples. As samples from metastatic sites could be difficult to obtain, another important challenge is the development of not invasive and reliable techniques able to provide genomic material for molecular studies. Correlation between tumor microenvironment, metabolic alterations and shift, as well as the identification of target and multi-targets inhibition strategies are key issues which will help us to better use our treatments, and to identify new targets and agents.

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Declaration of Competing Interest

All authors declare that they have no conflicts of interest

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