Cancer Drug Resistance

Cancer stem cells, plasticity and drug resistance

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Review

Cancer stem cells, plastic and drug resistance

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ABSTRACT

Melanoma is a highly aggressive tumor almost fatal when metastatic. Herein we discuss recent findings on the mechanisms of resistance of human cutaneous melanoma. To achieve a precision medicine approach, the heterogeneity and plasticity of tumor cells are two crucial aspects to be investigated depth. In fact, to understand the mechanisms that cells use to acquire a resistant phenotype after chemotherapy or how resistant cells inside the tumor are selected is the most important issue for a successful therapy. Since new therapeutic strategies are trying to go in this direction, we discuss here the state of the art of the research and the clinical impact of these strategies. We will also discuss and suggest further research development in the near future to define the best concentration and time of exposure of the drug or the cocktails of drugs for each specific patient because of his/her biological features.

Current therapeutic strategies for the treatment of human cutaneous melanoma

Melanoma arises from mutated melanocytes, the pigment producing cells. Although melanoma is a rare tumor, occurring in about 1% of all skin malignant tumors, it represents almost 2% of all cancer death worldwide[1], the survival rate is strictly related to the stage of the tumor and to the capability to perform an early diagnosis[2, 3] (Fig. 1). Furthermore, the age-adjusted rate of new cases reported in the USA between 1999 and 2016 shows an important increase of new cases of melanoma per year with respect to others kinds of cancer such as lung, breast and colon (Fig. 1). The overall survival is higher in the case of localized disease, but patients with metastatic melanoma show a very poor prognosis, with a median survival rate ranging from 3 to 6 months[2–4]. While low-grade primary tumor are usually successfully treated by surgical excision, systemic treatment of advanced metastatic disease treated with chemotherapy shows a low response rate and generally no overall survival rate improvement[5].

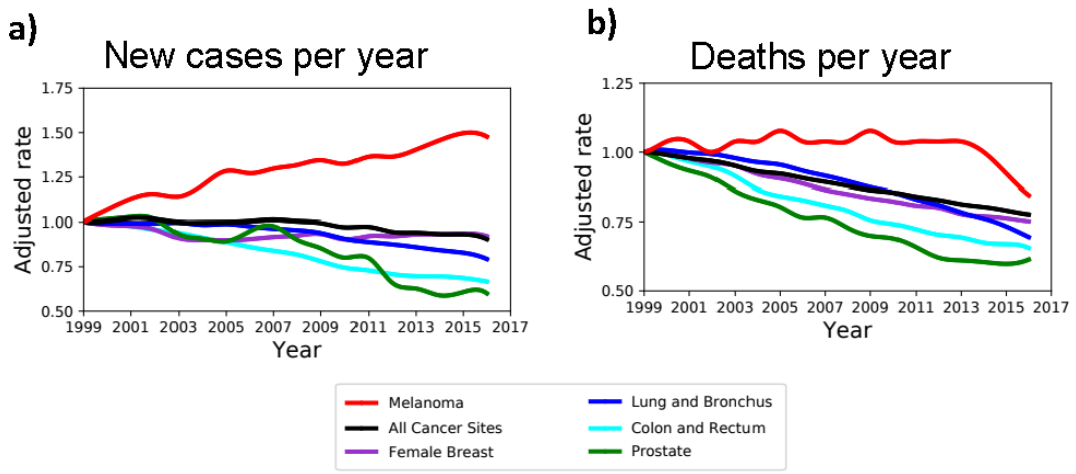


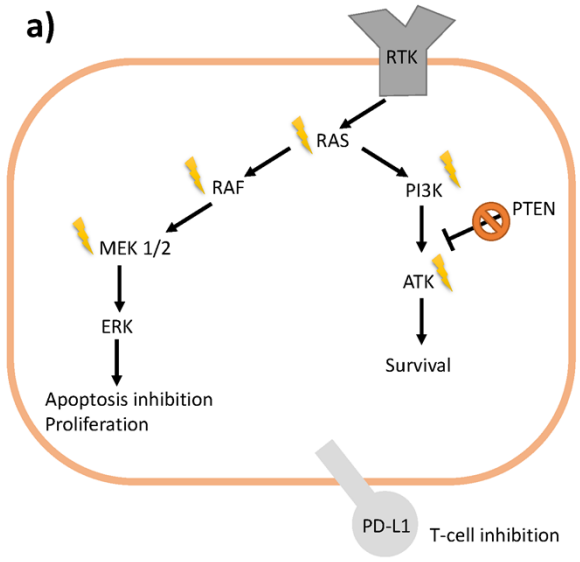
Figure 1. Melanoma incidence and death rate. a) Age-adjusted rate of new cancer cases diagnosticated in USA between 1999 and 2016 normalized over corresponding rate in 1999 for both sexes. Incidence is increasing in melanoma (red) compared to other type of tumors (colors as in legend). b) Age-adjusted death rate, for both sexes, between 1999 and 2016 in USA normalized over corresponding rate in 1999 for melanoma and top rated cancers by rates of cancer deaths.

Cutaneous melanoma is characterized by a series of peculiar somatic genetic alterations, frequently involving genes responsible for the control of cell cycle and proliferation, metabolism, growth and apoptosis that typically lead to the deregulation of mitogen-activated protein kinase (MAPK) and the phosphoinositol-3-kinase (PI3K)/AKT pathways[6, 7]. The most frequently mutated gene is BRAF, and in particular the missense mutation V600E is the most frequent mutation occurring in melanoma[8–10] while NRAS activating mutations was detected in small percentage of this tumor cases[8, 11]. BRAF is a serine-threonine kinase involved in the RAF-MEK-MAPK pathway controlling through ERK1/2 cellular proliferation, survival and differentiation[19]. Notably, NRAS and BRAF mutations are generally mutually exclusive; only in a minor proportion of patients the coexistence of both genetic alterations is reported[9, 12]. Additionally, genetic alterations of TERT promoter and CDKN2A or PTEN loss-of-function have been frequently observed in advanced melanoma[13–18].

Current therapeutic approaches of cutaneous melanoma include surgical resection, chemotherapy, photodynamic therapy, immunotherapy, biochemotherapy and targeted therapy depending on the features of the tumor such as its localization, stage and genetic profile. Chemotherapy combinations have been shown to improve the clinical response, however the overall survival does not change significantly[20]. Dacarbazine, approved in 1974 by FDA, is the standard drug used for metastatic melanoma. Temozolomide, which is an oral pro-drug of the active metabolite of dacarbazine, is used in advanced melanoma and it seems to improve the median progression free survival but not the overall survival[21, 22]. Electrochemotherapy is a technique that combines the use of cytotoxic drugs such as bleomycin and cisplatin, with high -intensity electric pulse which would facilitate the drug deliver inside the cells[23, 24]. Light-based therapy is a promising adjuvant therapy useful for palliative treatment in advanced metastatic melanomas[25].

Immunotherapy is mainly based on the frequent presence of chronic inflammation and of immune cells inside the tumor[26]. The possibility to target the immunogenic tumor microenvironment is nowadays one

of the more promising strategies for a successful cancer treatment. Regarding cutaneous melanoma, there are immunotherapies approved by FDA (i.e. nivolumab, pembrolizumab, gp100 vaccine). Nivolumab and pembrolizumab, approved for the treatment of metastatic melanoma, are anti-PD1 antibodies that block the interaction between PD-1, which is a membrane antigen and its receptor PD-L1/PD-L2. The blockade of the interaction between this ligand and its receptor induces antitumor activity showing a reduction of tumor progression through the modulation of the immune system[27]. Another interesting drug is ipilimumab which is an anti-CTLA-4 antibody that acts as receptor antagonists enhancing pro-inflammatory T-cell cytokine production and promotes clonal T-cell expansion[28, 29]. In Fig. 2 we report a scheme of the pathways on which those drugs work.



b)

Class of drug	Name	Resistance mechanisms
BRAF inhibitors	Verumafenib Dabrafenib Encorafnib	BRAF alternative splicing BRAF dimerization CRAF expression YAP upregulaton RTK - AXL upregulation
MEK inhibitors	Trametinib Cobimetinib Binimetinib	
Immunoterapy Anti CTLA4 Anti PD1/PD-L1	Ipilimumab Nivolumab	HLA class I negative phenotype

Figure 2. Melanoma therapies and resistance patterns. Fig. 1 a)Simplified schematic of the key molecular component of MAPK and PI3K–Akt signaling pathway related to melanoma tumorigenesis. Advanced-stage melanoma can be categorized according to mutational profiles. The main mutated genes and frequencies of key effectors are indicated, these include mutations in either the BRAF, RAS, or PTEN genes in approximately 50%, 20%, and 10% of patients. b)Targeted inhibitors: representative drugs and therapies and main resistance mechanisms.

Gp100 is a glycoprotein expressed by melanoma cell only with few exceptions (healthy epidermal melanocytes and retina) and it is recognized by cytotoxic T cells (CTL). The administration of gp100 epitopes enhances CTLs activity, however it is reported to have a limited clinical benefits and it is used as adjuvant therapy only[30].

Biochemotherapy is a combination of chemotherapy and immunotherapy. In fact, some conventional chemotherapies may act partially through immune-stimulatory mechanisms[31]. The most common use of biochemotherapy is the combination of dacarbazin, cisplatin and vinblastine with IL-2 and IFNa2b as immunoregulator.

Most of cutaneous melanoma are treated with targeted therapy, since about 70% of these tumors express specific mutations related to key signaling pathways (i.e. BRAF V600E) (Fig. 2). The targeted

therapy with the use of small molecules inhibitors or antibodies affecting these mutated proteins, which play a critical role for the progression of the tumor (Fig. 2) will be discussed in the next section.

Genetic and epigenetic mechanisms of resistance

Melanoma is a highly resistant tumor. The appearance of resistance after chemotherapy or the presence of intrinsic resistance, leads to a great difficulties in devising an effective and durable therapy and at the end to a poor survival of the patients in particular when they are already metastatic. In the last years many studies tried to understand the molecular basis of resistance. We herein discuss the main biological mechanisms displayed by melanoma to become resistant to the current therapies.

Treatment of advanced BRAFV600E mutant melanoma using a BRAF inhibitor or its combination with a MEK inhibitor, typically elicits only partial response. It has been reported that new genetic alterations arise in patients carrying BRAF mutation when treated with anti-BRAF antibody as well as in patients displaying both BRAF and MEK mutations and treated with inhibitors for both factors[32, 33]. In particular it has been reported that one of the main mechanisms of resistance is the reactivation of MAPK signaling[33]. Moreover, in a recent paper, the comparison between the transcriptomes of melanoma patient-derived tumors regressing after MAPK inhibitor (MAPKi) treatment with respect to MAPKi-induced temporal transcriptomic states show that residual melanoma on MAPKi therapy displays an adaptive transcriptomic, epigenomic and immune-regulomic alterations[34].

Hannan and coworkers observed that a non correct analysis of melanoma polyclonal population affects the choice of the therapy[35]. This aspect is relevant for melanoma since the drug therapy is usually applied when the disease is in advanced state[2]. The deletion or loss of function of PTEN, is also quite common in drug-resistant melanoma, reactivating PI3K-ATK pathway in a MAPK-independent manner[36–38]. On the other hand, transient resistance can be induced by compensatory changes in gene expression such as the upregulation of the receptor of tyrosine kinases, the overexpression of CRAF or the amplification or truncation of BRAF gene[37, 39–41].

The high heterogeneity of the tumor cells as well as their plasticity lead to the possibility that the same drug might induce the switch towards slow-cycling resistant phenotype associated to high MITF levels and to a mesenchymal-like phenotype[42–46]. Early adaptation involving a transcriptome reprogramming seems to be particularly relevant even at long time scale, allowing the tumor to survive until a genetic mutation and permanent resistance mechanism is acquired[43, 47]. Interestingly, melanoma cells can display profound transcriptional variability at the level of single cell that can involve the transcription of a number of resistance markers at high level in a very small percentage of cells[48]. The presence of a drug can, therefore, induce an epigenetic reprogramming in these cells converting the transient transcriptional state into a stable one[48].

Other important actors of drug resistance in melanoma are non-coding RNAs[49–51]. In this context, the use of combined and coadjuvant therapies have been proposed to avoid successive treatments failure due to the acquisition of a cross-resistance or changes in tumor environment[52–57]. Moreover, the tumor niche can play an important role and a long-term success of targeted therapies seems to be strictly related to a favorable microenvironment and immunologic signature[54, 58, 59]. In this connection, a recent paper shows that the development of drug resistance to anti-BRAF treatment is dominated by a

dynamic deregulation of a large population of miRNAs[60]. The latter leads to the alteration of the intrinsic proliferation and survival pathways enhancing proinflammatory and proangiogenic cues[60].

Role of immunity in resistance

Chronic inflammation is an hallmark of cancer[61–63]. Innate and adaptive immune responses contribute to select aggressive clones, stimulating cancer cell proliferation and migration[64]. NKs and cytotoxic T cells (CTL) can recognize and eliminate the immunogenic cancer cells and in this way less immunogenic cells are selected[65]. Tumor associated macrophages (TAMs) and neutrophils (TANs) can also promote angiogenesis and lymphangiogenesis as well as cancer cell proliferation and EMT by secreting set of stimulating cytokines[66–68]. On the other hand, the same tumor cells can secrete immunosuppressive factors, controlling the immune response[68–70]. Tumor-associated endothelial (TECs) cells also contribute to make cancer physically inaccessible to the immune system by increasing deposition of factors conferring, on one side, a higher stiffness of the extracellular matrix and, on the other hand, preventing immune infiltration in the tumor tissue and favoring tumour cell proliferation[69, 71]. In the light of these findings, several different immunotherapeutic strategies have been developed. Cytokines with immunomodulatory, antiangiogenic, anti-proliferative and antitumor activities, such as IFNs and IL-2, have been combined with chemotherapy however with less satisfying results[72]. Immune-checkpoint inhibitors, a class of target-specific drugs which interfere critical inhibitory signaling pathways promoting immune-mediated terget of tumor cells (i.e. Ipilimumab and nivolumab), gave more successful results[73].

Adoptive T-cells transfer therapy is, at the moment, one of the personalized and effective treatment method available for the management of metastatic melanoma. In this case, tumor-infiltrating lymphocytes (TILs) directly derived from the patients or genetically engineered melanoma-specific T-cells are expanded ex-vivo and then injected into the patients[74]. Although the complex anti-tumour mechanism triggered by this therapeutic approach has not yet been fully elucidated, the obtained results are very promising. Adoptive T-cells transfer therapy has been reported to be associated with complete and durable responses also in metastatic melanomas[75]. This approach results effective not only alone but also in combination with other standard therapies for melanoma management[76].

Phenotypic plasticity and drug resistance

Cancer is highly heterogeneous. This fact brings many important consequences: there is a profound variation between different individuals with the same type of cancer and, on the other hand, there is a high grade of genetic and phenotypical variability in the cancer cell population of a specific subject. The different phenotypes of tumor cells are due not only to genetic and epigenetic intratumor heterogeneity[77] but also to epigenetic changes due to the impact of the environment. It has been reported that genetically homogeneous tumor cells show a remarkable diversity with respect to either the therapy response or other environmental stimuli[78, 79]. Epigenetic gene regulation at molecular level from DNA methylation, post-translational modification of histones, non coding RNAs and chromatin remodeling are the most common mechanisms contributing to cellular epigenetic heterogeneity. For a cancer, the robusteness of the system is the capability to small cells to adapt to another environment, having the possibility to evolve new cellular ecosystems. The capability of cancer cells to adapt to critical changes in the environment leads to the difficulty in finding a successful strategy. The genetic mechanisms that contribute mainly to the ability of cancer to adapt to different microenvironment is

genetic destabilization[80, 81]. Furthermore, differences in tumor cell metabolism as direct impact of genetic mutations and/or altered microenvironment, have a directly impact on epigenetic changes[82]. In this connection, our group demonstrates recently that human melanoma cells can change their phenotype expressing epithelial- mesenchymal transition (EMT) markers dynamically thanks to a complex network of miRNAs[83]. The direct and more important consequence of these findings is that the cells show an intrinsic capability to dynamically change the phenotype in dependence on the environment[83]. Similar results were published recently for breast cancer[84].

The impact of plasticity of tumor on drug resistance is a key factor and is crucial to develop new therapeutic strategies. In this connection, a recent interesting paper describes the dynamics of single melanoma cells after the treatment with a drug and shows that the cells reprogram to a stable resistant state[48]. The reprogramming involve the lost of SOX-10 which mediates differentiation and the activation of Jun-AP1 and TEAD[48].

Conclusion and perspectives

Plasticity of tumor cells including melanoma is a critical issue for a successful therapeutic strategy. The ability of tumor cells to change their status using epigenetic mechanisms in dependence by the environment, like the tumor niche, has been shown to play a critical role for the acquisition of a resistant phenotype in response to a specific drug. The perspectives at the light of these findings, that in our opinion will be crucial in view of a precision medicine treatment are: i) to know the epigenetc profile of each specific tumor of each specific patients before the treatment to start the best therapy; ii) to avoid the capability of the tumor cells to change their phenotype during the treatment acquiring a resistant phenotype, acting both at the level of the tumor cells and at the level of the tumor niche.

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Authors' contributions

Design and write the paper: MCL, MRF and CAMLP

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest

Ethical approval and consent to participate

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Consent for publication

Not applicable.

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UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE E POLITICHE AMBIENTALI
DEPARTMENT OF ENVIRONMENTAL SCIENCE AND POLICY

December 6th, 2019

Editorial Board,
Cancer drug Resistance
Dear Editors,

Enclosed please find our manuscript "Cancer stem cells, plastic and drug resistance" for the the special issue "Drug resistance and cancer stem cell".

Best regards,

Caterina La Porta

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