MicroRNAs and CDK4/6 Inhibitor Treatment

Subjects: Oncology

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Cyclin-dependent kinase 4/6 (CDK4/6) inhibitors have emerged as novel treatment options in the management of advanced or metastatic breast cancer. MicroRNAs are endogenous non-coding 19–22-nucleotide-long RNAs that regulate gene expression in development and tumorigenesis. Herein, we explored the predictive role of microRNAs in treatment with CDK4/6 inhibitors.

Keywords: microRNAs ; biomarker ; CDK4/6 inhibitors ; resistance ; breast cancer

1. Introduction

Cyclin-dependent kinases 4 (CDK4) and 6 (CDK6) are crucial mediators of cell cycle progression through G1/S checkpoint regulation. CDK4 and 6 form a complex with Cyclin D1, which catalyzes the phosphorylation of the retinoblastoma protein (RB) [1]. Phosphorylated Rb disassociates from E2F transcription factors and enables the expression of E2F-responsive genes, that are necessary for cell cycle progression. Recently, CDK4/6 inhibitors, palbociclib (PD0332991), ribociblib (LEE011) and abemaciclib (LY2835219), have emerged as new therapeutic options that target the above-mentioned signaling pathway. Specifically, CDK4/6 inhibitors reinstate the Rb-regulated suppression of cell division [1]. Palbociclib was the first CDK4/6 inhibitor to receive US Food and Drug Association (FDA) approval in postmenopausal hormone receptor-positive HR (+), epidermal growth factor receptor 2-negative HER (−) advanced breast cancer, based on the results of the PALOMA-2 Phase III trial [2]. Subsequently, ribociblib and abemaciclib were also approved in combination with endocrine therapy for HR (+), HER2 (−) advanced breast cancer [3][4][5]. Currently, CDK4/6 inhibitors are under investigation as promising therapeutic agents in a wide array of malignancies, although still at a preclinical or early clinical stage [6]. However, mechanisms of de novo or acquired resistance to CDK4/6 inhibitors were reported [7]. Loss of Rb, p16 overexpression or upregulation of the Cyclin E1 gene (CCNE1) were identified as potential mechanisms of resistance [7][8]. Approximately 10% of patients will exhibit primary resistance to CDK4/6 inhibitors, while an increasing number of patients will eventually fail to respond to treatment [7]. Therefore, there is an increasing need for biomarkers to identify non-responders and personalize treatment.

MicroRNAs are endogenous, non-coding 19–22-nucleotide-long RNAs that mediate a posttranscriptional negative regulation of gene expression [9]. The active miRNAs emerge from larger 60–110 nucleotide precursor transcripts that are cleaved by endoribonuclease Dicer. Complementary pairing of miRNA and the 3′ untranslated region (3′ UTR) of their target mRNA leads to mRNA degradation or inhibition of translation. A single miRNA molecule can target multiple mRNAs and, conversely, one mRNA can be the target of multiple miRNAs [9]. Consequently, miRNAs regulate multiple cellular processes, including cell proliferation, migration and apoptosis [10]. Deregulated expression of miRNAs is frequently linked to tumor progression. miRNAs can function as endogenous suppressors of target genes (e.g., miR-34, let-7, miR 200 family), or as oncogenes (e.g., miR-155, miR-17-5p, miR-21) [11][12][13]. All these miRNAs that are implicated in cancer development are known as “oncomirs” [14]. Indeed, miRNAs serve as key regulators of the genome by modulating up to one third of all cellular transcripts [9]. Consequently, these regulatory elements could be exploited as diagnostic, prognostic or predictive biomarkers, or even as therapeutic targets to suppress carcinogenesis.

2. Current Insights on MicroRNAs and CDK4/6 Inhibitor Treatment

Overall, we provide preclinical evidence that miRNAs are implicated in the response to CDK4/6 inhibitors. A number of these endogenous molecules seems to confer resistance to CDK4/6 inhibition by altering the expression of downstream target genes, such as CDK6 and Cyclin D1, or downregulating the TGF-β, EGF or mTOR signaling pathways. Conversely, other miRNAs have been associated with sensitivity to CDK4/6 inhibition by interfering with the c-myc/miR-29b-3p/CDK6 axis, eliciting senescence, regulating the expression of cell cycle regulatory genes, such as CCNE1, CDK6 and E2F3, or by reducing cyclin D1 expression. These findings are consistent with the dual role of miRNAs as tumor repressors or tumor promoters [11]. This role is achieved either by directly regulating cell growth and apoptosis or by indirectly targeting

other oncogenes or tumor suppressors that modulate cell survival. The vast alteration in the “miRNome” of cancer cells compared with their normal counterparts provides a rationale of increased or decreased response to CDK4/6 inhibitors [12]. The characterization of the miRNA signature of each tumor as a prognostic and predictive biomarker still remains a challenging idea. It should be noted, however, that there is only preclinical evidence of such association and the lack of clinical data is remarkable.

Numerous studies highlighted the link between miRNA expression and chemoresistance. MiR-134, miR-197, miR-490-3P, miR-663 and miR-622 were all diversely expressed in ovarian cancer cells resistant to paclitaxel treatment [15][16][17][18], while the miRNA fingerprint composed of six miRNAs (let-7e, miR-30c, miR-125b, miR-130a and miR-335) could be used as an effective biomarker to identify chemoresistant ovarian cancer cells [19]. miR-27a and miR-451 regulate drug resistance mediated by the MDR1/P-glycoprotein, contributing to an MDR phenotype in cancer cells [20]. miR-134, miR-379 and miR-495 affect the sensitivity of small cell lung cancer cells (SCLC) to chemotherapy, while upregulation of microRNA-451 sensitizes non-small cell lung cancer (NSCLC) cells to cisplatin [21][22]. miRNAs were shown to be involved in chemoresistance of various other neoplasms, including pancreatic cancer [23], hepatocellular carcinoma [24][25], cholangiocarcinoma [26] and esophageal cancer [27][28]. This phenomenon emerges from the robust alteration of various pathways and target genes. Activation of the JAG1-Notch1 signaling pathway [29], EZH2 downregulation [24], Akt signaling pathway upregulation [27] or PTEN downregulation are some of the suggested mechanisms of miRNA-mediated chemoresistance.

Apart from their involvement in response to chemotherapy, miRNAs were shown to mediate the development of resistance to endocrine treatment in luminal breast cancer. Multiple miRNAs modulate estrogen receptor (ER) expression; therefore, they confer resistance to endocrine treatment in breast cancer cells [30][31][32]. miR-221/222, miR-342-3p, miR-873 and Let7b/Let-7i were found to downregulate ERa protein expression and lower ERa levels to serve as a mechanism of resistance [33]. More specifically, luminal breast cancer cells characterized by miR-221/222 overexpression are marked by greater aggressiveness (high Ki67 proliferation index and tumor grade) [33] and higher rates of acquired resistance to selective estrogen receptor downregulator (SERD) fulvestrant [32]. Consistently, overexpression of miR-221/222 rendered breast cancer cells resistant to tamoxifen treatment via targeting p27Kip1 [31]. miRNAs also modulate multiple transcription factors that interact with ERa, including FOXM1, NFκB and ERa coactivator nuclear receptor co-activator 3 (NCOA3) [33]. Indeed, miR-30c-5p, miR-30b-5p, miR-182-5p and miR-200b-3p were found to be independent predictors of clinical benefit from endocrine therapy [34]. It should be thus postulated that certain miRNAs may be involved in both CDK4/6 inhibitor and endocrine therapy resistance in luminal breast cancer. Downregulation of ER and activation of the PI3K/Akt/mTOR and CDK4/6/RB pathways are some of the mechanisms of resistance to both endocrine treatment and CDK4/6 inhibitors that could be affected by miRNAs.

Despite the recent establishment of CDK4/6 inhibitors in clinical practice, mechanisms of resistance were already described [7][8]. The amplification of cell cycle regulators, such as p16, CDK6, CCNE1/2, CDK2, CDK7 and E2F transcription factors, plays a key role in de novo and acquired resistance to CDK4/6 inhibitors. Given the strong link between miRNAs and the expression of these cell cycle genes, it could be postulated that antitumor efficacy of CDK4/6 inhibitors could be mitigated by certain miRNAs. However, the interaction of miRNAs with cellular processes, such as cell cycle progression, apoptosis and migration, tends to be more complex, so miRNAs can have both a negative and a positive effect on CDK4/6 inhibition. Since there is a growing attention to identify biomarkers of response to CDK4/6 inhibitors, miRNAs could be used as a valuable alternative.

Another significant aspect of determining the exact relationship between miRNAs and CDK4/6 inhibition is the therapeutic potential they provide. An increasing number of studies utilizes miRNAs to design targeted therapies, such as miRNA inhibitors (antagomirs) or miRNA mimics, to inverse their effect on carcinogenesis [35][36][37][38][39]. NOV340/miR-34a is a novel liposome miR-34a-containing particle that was tested in murine models bearing liver tumors [39]. This novel agent targeting miR-34a was tested in Phase I trial in solid tumors and hematological malignancies (NCT01829971), although the study was prematurely ended due to immune-related adverse events [40]. Moreover, novel compounds, namely, “antagomirs”, that silence miRNAs in vivo were effective in downregulating miRNA expression in mice [37]. “Antagomirs” could be given as a monotherapy or in combination with conventional chemotherapeutic drugs, e.g., doxorubicin, in order to maximize the antineoplastic effect achieved [36]. Once the interaction between miRNAs and CDK4/6 inhibitors is adequately clarified, new treatment strategies combining miRNA-targeting agents and CDK4/6 inhibitors will become feasible.

Among the limitations of this systematic investigation, it should be noted that the study selection process was essentially driven by the search algorithm, which focused primarily on titles and abstracts of the published literature, in order to provide more relevant results. Additionally, clear heterogeneity was observed in our findings, due to differences in

malignancies, isolation protocols, detection methods and sample types (i.e., formalin-fixed paraffin-embedded tissues, freshly frozen tumors, cell lines); thus, estimating the pooled effects by performing a meta-analysis was not a feasible task. Lastly, the number of studies on microRNAs associated with response to CDK4/6 inhibitors is still limited, since we are referring to a relatively new treatment modality, suggesting that further studies should be conducted to verify the abovementioned observations.

3. Conclusions

Collectively, we demonstrated that miRNAs mediate the function of CDK4/6 inhibitors and are often associated with drug resistance. Further investigations are warranted to enlighten the impact of miRNAs on CDK4/6 inhibition and to exploit this association as a novel therapeutic target, since only preclinical evidence is available until now.

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