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論文内容の要旨

Resistance of cancer cells to drug treatment is a major problem in cancer therapy. The platinum-based drug cisplatin (cis-diamminedichloroplatinum(II)) is a widely used anticancer drug introduced more than 20 years ago which acts mainly by forming adducts with DNA that cause the induction of apoptosis. However, some types of cancer have high intrinsic resistance to cisplatin; in addition, resistance to the drug frequently appears in initially responsive tumors. The mechanisms of resistance to cisplatin are the subject of intense study because their elucidation will lead to the development of better, more effective treatments for cancer.

A model of cisplatin resistance is provided by the cisplatin-resistant KB (KB/CP4) cell line, which is derived from human KB epidermoid cancer cells. It was found previously that activity of the volume-sensitive, outwardly rectifying (VSOR) chloride channel is virtually absent in these cells. Because VSOR chloride current activation is known to be essential to the progression of apoptosis, it was hypothesized that the absence of the current contributes to the cisplatin resistance of these cells.

An attempt was first made to determine the molecular identity of the VSOR chloride channel or a regulator of it. A strategy for identifying the molecule(s) was suggested by the fact that the KB/CP4 cell line, unlike the KB cell line from which it is derived, does not express volume-sensitive chloride current. Using a subtractive hybridization method, candidates for differentially expressed genes were identified. With semi-quantitative RT-PCR, seven genes were confirmed as being expressed at a higher level in KB cells than in KB/CP4 cells; five of these were genes which had been identified by subtractive hybridization. The genes confirmed as having higher expression in KB cells were lactate dehydrogenase A (LDHA), S100 calcium binding protein A4 (S100A4), thymosin „4 (TMSB4X), argininosuccinate synthetase (ASS), placental alkaline phosphatase (ALPP), folate receptor 1 (FOLR1) and GPI-specific phospholipase D1 (GPLD1). All of these genes, except for ALPP, were then cloned into expression vectors and overexpressed in KB/CP4 cells to determine if they could restore VSOR chloride current expression. The overexpression of these genes did not result in restoration of the current in KB/CP4 cells, however.

Because VSOR chloride current could not be restored in KB/CP4 cells by gene overexpression, treatment with drugs that remove gene silencing was tested in an attempt to restore the current. Epigenetic changes which alter chromatin structure--gene promoter methylation and histone deacetylation, in particular--are known to cause inhibition of gene expression, and could potentially contribute to the silencing of genes in KB/CP4 cells. 5-aza-2'-deoxycytidine (Aza-dC) is a drug that reverses DNA methylation by inhibiting DNA methyltransferases, and trichostatin A (TSA) is a drug that reverses histone deacetylation by inhibiting histone deacetylases (HDACs). In KB/CP4 cells treated with Aza-dC, a volume-sensitive chloride current was not induced; however, it was found that the current could be induced in cells treated with TSA. This current was confirmed as VSOR chloride current based on its volume sensitivity, intracellular ATP dependency and sensitivity to two blockers, 4,4'-diisothiocyanatostilbene-2,2'-

disulfonic acid (DIDS) and phloretin. It was found that in addition to this phenotypic change in the expression of VSOR chloride current, alterations in the expression of some genes occurred due to TSA treatment. As assessed by semi-quantitative RT-PCR, two genes previously identified as differentially expressed in KB and KB/CP4 cells, FOLR1 and GPLD1, were upregulated in TSA-treated KB/CP4 cells.

The effects of restoration of VSOR chloride current on cisplatin resistance in these cells were checked by a cell viability assay and caspase-3 activity assay. The viability of cells treated for 48 h with both TSA and cisplatin decreased to a greater extent than that of cells treated with TSA alone, and this decrease could be blocked by treatment with DIDS. Moreover, simultaneous treatment with both TSA and cisplatin for 24 h was found to induce apoptosis in this cisplatin-resistant cell line: caspase-3 activity in cells treated for this length of time with both drugs was elevated compared to that in cells treated with TSA alone. This increase in caspase-3 activity could also be blocked by DIDS. These results demonstrate that cisplatin resistance in KB/CP4 cells treated with the HDAC inhibitor TSA decreases as a result of an increase in apoptosis, at least in part; in other words, TSA caused a sensitization of the cisplatin-resistant tumor cells to cisplatin-induced apoptosis. It is thus suggested that the activity of the VSOR chloride channel is essential for the TSA-induced increase in apoptosis.

In conclusion, this study provides evidence that the absence of VSOR chloride channel activity contributes to cisplatin resistance in a cisplatin-resistant KB cell line, and that restoration of VSOR chloride current in these cells is involved in sensitization to cisplatin-induced apoptosis in this tumor cell line. The identities of genes involved in the functioning of the channel remain to be clarified.

論文の審査結果の要旨

LEE 氏の属する研究室では、これまでヒト扁平上皮細胞由来の細胞株である KB 細胞を抗ガン剤のシスプラチンの薬理作用に関する研究に用いてきた。この細胞に発現する VSOR(容積感受性クロライドチャネル)がシスプラチン感受性に関与していることが示唆されてきた。LEE 氏は、KB 細胞のサブクローンである KB/CP4 細胞がシスプラチンに対して感受性を持たないことに注目し、この細胞での VSOR の機能とシスプラチン感受性との連関を、ホールセルパッチクランプ法による電気生理学的解析と、遺伝子発現パターンの解析を組み合わせて総合的に明らかにする研究を行った。更に、まだ VSOR の分子実体の明らかでないことを背景に、KB/CP4 細胞と KB 細胞における VSOR 電流量の相違に着目し、VSOR そのものの分子機構を明らかにする実験も試みた。

ホールセルパッチクランプ法の結果、KB 細胞では著明な VSOR 電流が記録されるのに対して、KB/CP4 細胞ではほとんど VSOR 電流が検出されなかった。クロモゾームレベルでの遺伝子発現の抑制を解除する薬剤であるトリコスタチンを投与したところ、マーカー分子(FOLR1 と GPLD1)の遺伝子発現の抑制が解除され、KB/CP4 細胞において KB 細胞の三分の一程度の VSOR 電流が出現するようになった。

VSOR 電流の出現に伴い、シスプラチン感受性が出現するかどうかを検討するため、トリコスタチンとシスプラチン存在下において細胞生存率とカスパーゼ3の活性を測定したところ、シスプラチンに対する感受性が回復される効果が確認された。

本研究結果は、KB/CP4 細胞がシスプラチンに対して抵抗性を示す原因が VSOR を発現していないことに起因することを示しており、これまでのシスプラチンによる細胞死の誘導に VSOR が必要であるという考えを確認した。また遺伝子発現パターンの解析の結果は VSOR の分子実体を明らかにするには到らなかったが、VSOR の発現制御についての知見を得ることに成功し、今後シスプラチン耐性に関わる遺伝子発現カスケードについて理解する上で有用な基盤を得るに到った。

以上のように LEE 氏の論文は学位取得に十分値すると判断された。