Genomic differential expression analysis of fusion proteins incorporating the pro-apoptotic molecule Granzyme B reveals new potential targets for treatment of breast cancer

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Abstract

Granzyme B (GrB) is a member of the serine protease family of enzymes that play a critical role in the body's defense against viral infection and tumor development. Cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells directly deliver granzymes to target cells, which induces apoptosis through both caspase-dependent and caspase-independent multiple-cascade mechanisms. Anti-tumor efficacy studies have suggested that the targeted delivery of human Granzyme B to tumor cells has a significant potential for cancer treatment. We had previously developed a novel fusion construct composed of the VEGF121 growth factor and human GrB. The GrB-VEGF121 construct was found to live highly cytotoxic to vascular endothelial cells expressing the KDR (Flk1) receptor for VEGF. In this study, we examined the mechanism of GrB-VEGF121-induced cytotoxicity against cells in culture at the genomic level. The TNBC cell line MDA-MB-231 was treated with an IC50 dose of GrB-VEGF121 for 24 hours; the cells were then harvested and the effect of GrB-VEGF121, on intracellular events was examined by extraction of mRNA followed by microarray analysis. Gene-level differential expression analysis revealed that a total of twenty genes were upregulated by over 3-fold, while twenty-five were downregulated at 3-fold or more. These included genes involved in signal transduction, stress response, cell cycle control, hypoxia and metastasis. Validated data will be reported following complete analysis of gene level differential expression and alternative splicing. Our data suggests that GrB-VEGF121 induces expression of genes known to be induced by VEGF alone, as well as molecules previously not associated with either Granzyme B or VEGF. This data suggests a previously unsuspected impact of the serine protease GrB on various molecular pathways within the cell and may lead to a new understanding of how these agents operate at the molecular level. Research conducted, in part, by the Clayton Foundation for Research.

GrB/VEGF121

• 80 kDa homodimer (disulfide linked) fusion toxin composed of VEGF121 and the serine protease Granzyme B
• VEGF121 binds only to VEGFR-1 (Flk-1/Flt1) and VEGFR-2 (Flk-1/KDR)
• Mechanism of action of Granzyme B is well characterized but its impact at the genomic level is not known

Isolation of total RNA to probe the pathways targeted by Granzyme B

Gene expression changes in MDA-MB-231 cells by 24 h treatment with GrB/VEGF121

Genes upregulated or downregulated by > 3-Fold

Significant Pathways

Summary

• Dot laboratory has developed a novel fusion construct composed of the VEGF121 growth factor and human pro-apoptotic protein Granzyme B.
• We examined the mechanism of GrB-VEGF121-induced cytotoxicity against cells in culture at the genomic level by treating the TNBC cell line MDA-MB-231 with an IC50 dose of GrB-VEGF121 for 24 hours.
• Gene-level differential expression analysis revealed that a total of twenty genes were upregulated by over 3-fold, while twenty-five were downregulated at 3-fold or more.
• Twenty-five pathways were identified to have been significantly impacted by GrB/VEGF121 treatment.
• For most of the pathways that have been significantly impacted by GrB/VEGF121 treatment, gene expression in those pathways was down-regulated rather than up-regulated.
• Most genes with a change in expression of 3-fold or more belong to pathways that are not yet associated with either potential Granzyme B targets or VEGFR1/2.
• Further elucidation of the impact of Granzyme B on various molecular pathways may lead to a new understanding of how these agents operate at the molecular level.