Comparative Efficacy Study of IL-15 Transfections in Various Mouse Cancer Cells (RM-1 Prostate Cancer, B16 Melanoma, and 4T1 Breast Cancer)

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Introduction

Cancer is a major cause of morbidity and mortality around the world. Despite surgical techniques, radiation therapy, chemotherapy, and biological therapy, approximately 50% of all cancer patients will die from their disease. In addition, it is estimated that more than one million Americans will develop cancer every year (1). Due to the high diagnosis and death rates new prevention and treatment regimens are needed. One such solution is the development of cancer vaccines.

Early stages of the vaccine development in mice involved the treatment of B16 cancer cells with interferon-α (IFN-α) in vitro for one week (2). When lethally irradiated B16α cells were injected into mice, without an adjuvant, immunity developed to the parental tumor cells (2). The immunity required CD4 helper T cells, macrophages, CD8 cells, and Natural Killer cells (3). In addition, there was a profound activation of lymphocytes that persisted for weeks after vaccination (4).

IL-15 induction by IFN-α was found to be associated with the vaccine efficacy (4). To discover whether IL-15 was inducing the immunity, a specific interfering RNA for IL-15 (siRNA-IL-15) was cloned into a vector, blocking the accumulation of cell-associated IL-15 (Unpublished observation). As a control, a scrambled RNA sequence (scrRNA) with no known activity was also cloned into a vector. The two vectors were then separately cloned into B16 cells and treated with IFN-α, producing B16α cells. Vaccination with B16α and B16α-scrRNA cells showed similar immunity. However B16α-siRNA cells did not induce immunity, demonstrating that the depletion of IL-15 eliminates the vaccine efficacy of B16α cells (4).

Although the vaccine protocol worked in mice, the IFN-α treatment was very expensive. Furthermore, IFN-α treatment of human cells did not give high levels of IL-15. Thus, another avenue was explored, leading to the transfection of B16 cells with the IL-15 genes. In doing this an important question was asked; was the IL-15 secretable or non-secretable? To answer this question, originally two splice variants were used; IL-15 with a long peptide leader sequence (IL-15 LSP) and IL-15 with a short peptide leader sequence (IL-15 SSP). According to the literature (3), the IL-15 LSP produces IL-15 which is secreted from the cell, whereas IL-15 SSP produces IL-15 which accumulates in the cell. Through Real-Time RT PCR and ELISAs studies, it was determined that no secreted IL-15 could be detected in cultures of B16 cells transfected with IL-15 LSP. A high level of cell-associated IL-15 was detected in B16 cells transfected with IL-15 SSP. However, when this work was extended to other cancer lines such as RM-1 prostate cancer and 4T1 breast cancer, it was found that there was down-regulation of the IL-15 genes in these cancer cell lines.

An IL-15 genetic variant containing a very short peptide leader sequence (IL-15 VSSP) was developed to try to produce higher levels of cell-associated IL-15. RM-1, B16, and 4T1 cells were transfected with the various IL-15 genetic constructs (IL-15 LSP, IL-15 SSP, and IL-15 VSSP) and monitored for IL-15 production. B16 cells transfected with the various IL-15 genetic constructs were evaluated for vaccine efficacy.

References

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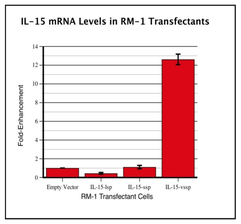
Results

1.



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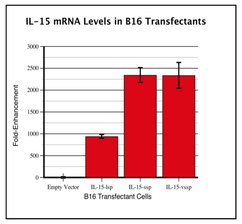

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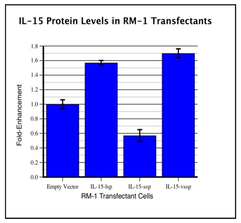

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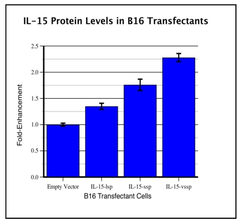

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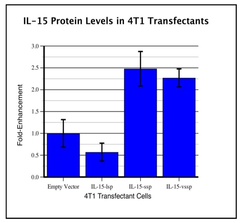

Figure 1 & 2: Near confluent to confluent monolayers of RM-1 and B16 transfectant cells were harvested at one day after receiving fresh media. The cells were lysed, and the cell extracts were subjected to Real-Time RT-PCR. The levels of IL-15 mRNA and GAPDH mRNA were determined. After adjusting for the levels of GAPDH mRNA in each of the preparations, the level of IL-15 mRNA in the RM-1 empty vector was set at 1, and the levels of IL-15 mRNA in the RM-1 and B16 transfectant cells were expressed as fold enhancements (2).

Figure 3, 4, & 5: Near confluent to confluent monolayers of RM-1, B16, and 4T1 transfectant cells were harvested at two days after receiving fresh media. After washing the monolayers three times, the cells were harvested, pelleted by centrifugation, resuspended, and sonicated (2). The sonicates were assayed for the presence of IL-15 protein with ELISA. Standard curves of IL-15 were also run, and after comparison to the IL-15 standard curve, the results were expressed as picograms of IL-15 per 10^6 cells of cytoplasmic IL-15.

Vaccine Efficacy in B16 Transfectant Cells

Vaccine Cells	Exp. 1	Exp. 2	Total
1. B16 cells transfected with empty vector	0/10	0/10	0/20
2. B16 cells transfected with IL-15 LSP	1/10	0/10	1/20
3. B16 cells transfected with IL-15 SSP	2/10	2/10	4/20
4. B16 cells transfected with IL-15 VSSP	4/10	4/10	8/20

Table 1: The indicated transfected vaccine cells were lethally irradiated and i.p. inoculated (10^6 cells/mouse) four times at weekly intervals. Live B16 cells (10^6 cells/mouse) were i.p inoculated as a challenge. The mice were monitored for survival. Group 1 vs. Group 2: p=NS; Group 1 vs. Group 3: p=0.053, Group 1 vs. Group 4: p=0.0016; Group 2 vs. Group 3: p= NS; Group 2 vs. Group 4: p=0.0092.

Conclusions

B16 and RM-1 cells were treated with interferon to create alpha cells. RM-1α cells appeared to show only a marginal increase in the production of IL-15, as compared to a high production by B16α cells (4). This finding led to the creation of IL-15 transfectant cells to boost the production of IL-15. RM-1 transfectants showed significantly lower levels of IL-15 mRNA synthesis than B16 transfectants. This observation suggested a down regulation of the transcription IL-15 message in RM-1 cells. Of the RM-1 IL-15 transfectants only the IL-15 VSSP showed a significant enhancement in the transcription of the IL-15 message. The difference between the RM-1 IL-15 SSP and IL-15 VSSP involves a deletion of upstream bases in the IL-15 gene. The observation that IL-15 VSSP gave good transcription suggests that IL-15 VSSP must have a deletion of an upstream transcriptional regulatory sequence. This concept is supported by the observation that B16 cells are permissive of IL-15 transcription and the level of IL-15 VSSP transcribed is not enhanced over the transcription of IL-15 SSP.

It has been previously suggested that there is an upstream transcription regulatory sequence (6). Indeed, the differential levels of RM-1 transcription have been reported previously for RM-1α cells in Wu and Fleischmann (5). Data with our transfected RM-1 cells further supports this concept.

- For RM-1 and B16 cells, there is greater IL-15 mRNA production in IL-15 VSSP transfected cells then in the IL-15 SSP transfected cells. It is known that the Kozak initiation sequence is located in a double stranded environment in IL-15 SSP mRNA but in a single stranded environment in IL-15 VSSP mRNA. This suggests that RM-1 and B16 may have difficulty recognizing the double stranded initiation region of IL-15 message. Furthermore, the regulatory event that occurs in RM-1 and B16 cells that prevents efficient recognition of the Kozak sequence when it is in the double stranded orientation does not occur in 4T1 transfectant cells. These findings suggesting that the Kozak sequence of IL-15 can be effectively recognized by 4T1 transfectant cells whether it is in double stranded or single stranded orientation.

In RM-1, B16, and 4T1 transfected with IL-15 LSP, IL-15 SSP, and IL-15 VSSP the IL-15 protein levels were monitored. It was noted that IL-15 translation in cells transfected with IL-15 LSP was relatively higher in RM-1 cells then in B16 cells. The Kozak sequence is present in a region of single stranded region of mRNA in IL-15 LSP. So, the location of the Kozak sequence would not be the expected reason for this differential expression. There must be some other, as yet undefined, differential regulation of IL-15 LSP versus IL-15 VSSP mRNA translation.

In regards to the production of IL-15 protein the IL-15 VSSP transfectant was well translated in RM-1, B16, and 4T1 cell lines. For the RM-1 cell transfectants there was a good level of translation for the IL-15 LSP transfectant and slightly better translation with the IL-15 VSSP transfectant. However, translation of the IL-15 SSP transfectant was inhibited below detectable levels, for reasons stated above, making this cell line unique for the absence of translation of IL-15 SSP transfectant.

There was an upward trend in IL-15 production from IL-15 LSP, IL-15 SSP, and finally IL-15 VSSP in B16 cells, with each progressive transfectant producing more IL-15 protein. Due to the significant levels of IL-15 produced for all three transfectants B16 was chosen as the vaccine model.

For 4T1 the IL-15 SSP and IL-15 VSSP transfectants produced similar amounts of IL-15 protein. However, the IL-15 LSP transfectant produced undetectable levels of IL-15 protein. It is not known why the IL-15 LSP transfectant doesn't show higher levels of IL-15 production, but since IL-15 VSSP has been determined to be the highest in all three cancer lines and the most possible for vaccine efficacy, the IL-15 LSP question is of less importance.

In the vaccine study there was no significant protection offered with the IL-15 LSP transfectant, but the IL-15 SSP transfectant did show a trend towards an efficacious vaccine with 20% protection. Lastly, the IL-15 VSSP transfectant produced significant levels of protection (40%), with 8/20 mice surviving the challenge with live B16 parental cells. IL-15 LSP gives rise to secreted IL-15 and is not effective as a vaccine. However, cell transfectants that produce cell-associated IL-15 (IL-15 SSP and IL-15 VSSP) are effective as a vaccine suggesting that the IL-15 VSSP transfectant will make the most effective vaccine for 4T1 tumor studies and in human studies.