Overexpression of IL-1 in human cells by using retroviral vectors

1. Aim of the project

Human interleukin-1 (IL-1), a pleiotropic cytokine, is known to be upregulated in many tumor types and has been implicated as a factor in tumour progression via the expression of metastatic and angiogenic genes and growth factors. Within the IL-1 family, there are two IL-1 agonistic proteins, IL-α and IL-β, derived from different genes but with similar functions. These proteins differ in the manner they are processed and secreted: IL-α is secreted intracellularly whereas IL-β is first cleaved into a mature form and then secreted extracellularly. A number of studies have reported that high IL-1 concentrations within the tumour microenvironment are associated with a more virulent tumour phenotype. Quantitative reverse transcription – PCR has shown a > 1000 fold higher gene expression of IL-α or IL-β in several tumor cell lines (Elaraj DM et al, 2006). The proteins are believed to act via induction of pro-metastatic genes such as matrix metalloproteinases and through the stimulation of adjacent cells to produce angiogenic proteins and growth factors such as VEGF, IL-8, IL-6, TNFα, and TGFβ.
This project involves the study of the role of IL-1 in cancer by overexpressing IL-β in human cells.

2. Vector and vector production system

• Vector : Replication deficient retroviral vectors, based on the Moloney murine leukemia virus (MoMLV, commercially available).
• Vector production system : For the production of the replication deficient vectors, a human packaging cell line HEK 293 (human embryonic kidney cells) is used. This cell line is stably transfected with *gag*, *pol* and *env* genes that are located on different constructs in such a way that three different recombination events are required to generate replication competent retroviral (RCR) particles (packaging cell line of the third generation). Various assays for RCR contamination all failed to detect the presence of RCR, underscoring the relative safety of such packaging systems of the third generation.
In this case the packaging cell line is cotransfected with an amphotropic viral envelope protein allowing the production of amphotropic replication defective vectors.

3. Insert :

The insert is a coding sequence for human interleukin-1β. This cytokine plays a key role in the inflammatory response of the body against infection. As mentioned above, IL-1 is also known to be involved in the up-regulation of many tumour types and has been implicated as a factor in tumour progression via the expression of metastatic and angiogenic genes and growth factors.

4. Localisation of inserted genetic material :

Stable integration into the genome

5. Receptor organism :

As the produced vectors are amphotropic, various types of cell may be transformed. In this case, a human non-tumorigenic epithelial cell line is (MCF 10A) is transfected with the recombinant vectors carrying the coding sequence for IL-1β.
For more information on the risk assessment of animal cell cultures (see http://www.biosafety.be/CU/animalcellcultures/mainpage.html)

6. Biological hazards and/or considerations related to the manipulation of the resulting GMO

Two types of GMOs are discussed in this example, namely 1) a recombinant replication defective vector carrying the coding sequence of IL-1 and 2) a human cell line (MCF 10 A) that is stably transfected with the recombinant vectors.

Considerations related to the production and the use of the recombinant vectors:

- Due to the use of third generation vectors, the probability of generating RCR is very low.
- Recombinant vectors are easy to inactivate and are normally not transmitted by air. Biological hazards during the manipulation of the recombinant vectors could be generated by accidental parenteral inoculation or exposure of broken skin to infectious aerosols.
- The recombinant vectors are amphotropic, various types of dividing cells can be infected (only dividing cells)
- Since integration is ad random, genomic integration can possibly occur in or near an oncogene sequence.
- Recombination events could possibly occur between human endogeneous retroviral sequences (which are inherently replication defective) and the transfected MoMLV based recombinant particles. However this is not expected to result in the formation of functional and replicatif competent recombinant viruses during vector production because cis-acting elements of the human endogeneous sequences are mainly incompatible with the proteins of the MoMLV based vectors.
- The possibility that genomic integration of the vector could occur in or near an oncogene sequence and the fact that transfection with the recombinant vector allows constitutive expression of a factor that is shown to promote tumour progression (IL-1) result in the fact that we are two steps closer to the possibility to form tumours.

Considerations related to the use of the transfected cells :

- Animal cells (genetically modified or not) hardly survive in non-optimized conditions of growth, that is in a hostile environment where control of temperature and osmolality is lacking or where cell-specific nutrients (e.g. glucose, vitamins, lipids) are not balanced or missing. Therefore, independent of the possibility that the genetic modification (overexpression of IL-1) could increase tumour progression, the survival of the transfected cell lines outside of proper conditions is unlikely to occur.
- Transfected cells are more likely to cause harm when entering the body of animals or humans. However, the extent of the harmful effect remains hard to predict. It should be kept in mind that the lack of histocompatibility between recombinant cells and the (accidental) host organism remains a major obstacle for these cells to survive and to multiply as the natural immune response of the healthy (non-immunocompromised) host will recognise foreign cells and eventually destroy them.

7. Class of risk of the resulting GM organism

Vector producing cells (HEK 293) and replication deficient vectors are of class of risk 3.
Transfected human cell lines may be considered as class of risk 2.

8. Class of risk of the activity

The activity involves standardized manipulations on laboratory scale. Taking account with the biological hazards linked to the GM organisms, the activity can be assigned to class of risk 2 (cfr Annex III of Directive 98/81/EC).

9. Recommended containment measures

Level of containment 2 with the additional use of a biosafety cabinet type II and adequate work practices in order to prevent accidental parenteral inoculation and exposure of broken or injured skin to infectious aerosols.

10. References and further reading

Elaraj DM *et al*. 2006. The role of interleukin I in growth and metastasis of human cancer xenografts. Clin Cancer Res 12, 1088-1096.

Lewis A et al. 2006. Interleukin-1 and cancer progression : the emerging role of interleukin-1 receptor antagonist as a novel therapeutic agent in cancer treatment. Review. Journal of Translational Medicine 4, 1-12.

Voronov E. et al. 2003. IL-1 is required for tumor invasiveness and angiogenesis. Proc. Natl Acad Sci USA 100, 2645-2650.