Review Article

Gene therapy as a treatment of oral cancer: An insight

Baishakhi Modak[1], Monica C. Solomon[1], Shaswata Karmakar[2], Shreyasi Polley[1]

[1]Department of Oral and Maxillofacial Pathology and Oral Microbiology, [2]Department of Periodontology, Manipal College of Dental Sciences, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India

Abstract

Oral cancer or oral squamous cell carcinoma comprises more than three-fourth of all the malignant neoplasms of the oral cavity. Worldwide, it is the 18th most common malignancy. The patients suffering from cancer usually remains immune to the standard therapies such as surgical resection of tumours, radiotherapy and chemotherapy; however, there can be probabilities of chronic and acute toxicities and secondary malignancies as well. Recently, *gene therapy* has been introduced in the arena of biomedicine to improve the treatment modality for oral malignant and potentially malignant disorders. It replaces the defective gene followed by repairing by a therapeutic gene. Gene therapy can attack cancerous cells without causing harmful effect to the normal tissue. It is useful to cope with the relapse of diseases and as a synergetic treatment. The present article reviewed the types of gene therapy, modes of delivery of the therapeutic genes and different techniques used along with pros and cons of gene therapy.

Keywords: Gene therapy, nanoparticles, oral squamous cell carcinoma (OSCC)

Address for correspondence: Dr. Monica C. Solomon, Dean and Professor, Department of Oral and Maxillofacial Pathology and Oral Microbiology, Manipal College of Dental Sciences, Manipal, Manipal Academy of Higher Education, Manipal - 576 104, Karnataka, India.
E-mail: monica.charlotte@manipal.edu

Submitted: 17-Sep-2022, **Revised:** 31-Jan-2023, **Accepted:** 24-Feb-2023, **Published:** 13-Jul-2023

INTRODUCTION

Oral cancer or oral squamous cell carcinoma (OSCC) is a genetic disease where there is mutation of the genes that control apoptosis and cell growth.[1] It comprises approximately more than three-fourth of all the malignant neoplasms of the oral cavity.[2] According to the literature, it is the 18th most common malignancy in the world. The aetiology of oral cancer mainly includes the use of tobacco and related products.[3] Individuals falling into their 5th decade of life or beyond are more susceptible to get affected by oral cancer. However, approximately 6% of the cases are also seen in individual below 45 years of age.[4,5] The patients suffering from cancer are usually un-wavered by the standard therapies such as surgical resection of tumours, radiotherapy and chemotherapy; but there can always be probability of chronic and acute toxicities and secondary malignancies as well.

Recently, gene therapy has been introduced in the arena of biomedicine to improve the treatment modality for oral cancerous lesions. The therapeutic gene repairs and replaces the defective gene.[6] According to the literature, the inactivation or activation of other genes may suppress or inhibit the growth of tumour by the introduction of new genes.[7] In gene therapy, cancerous cells are targeted without causing harmful effect to the normal tissue. This may be useful to cope with the relapse of diseases and also act as a synergetic treatment, for example, in resected tumour margins. Gene therapy can be used in the other fields of dentistry such as in the treatment of periodontal

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_46_23

How to cite this article: Modak B, Solomon MC, Karmakar S, Polley S. Gene therapy as a treatment of oral cancer: An insight. J Oral Maxillofac Pathol 2023;27:390-5.

disease, pain, auto immune diseases, caries and bone disorders.[8]

Archival standpoint

According to archives, Joshua Lederberg and Edward Tatum are known as the pioneers in the field of gene therapy. Their work got the limelight in the year 1944.[9] In 1977, Michael *et al*.[10,11] in their study successfully transferred TK gene, which codes for thymidine kinase in mammalian cells.[10,11] For the first time, Ashanthi De Silva in 1990 performed a clinical trial using gene therapy on a girl child having severe combined immunodeficiency syndrome (SCID). He extracted her T-cell, manipulated that with the retroviral vector carrying normal ADA gene and re-administered that on her.[12]

CONCEPT BEHIND GENE THERAPY

Gene therapy works on the notion of modifying the genetic material of a living cell. In other words, it is a process in which the faulty gene in an infectious cell genome is replaced to reimpose normal cell function and tissue integrity. Ideal requirements of gene therapy are:

- Easy delivery of the therapeutic material to the target cells.
- Genetic malfunction of a disease must be made understandable.
- Therapeutic material must remain active for the required duration.
- Therapeutic material must deliver the intended gene to the target cells.
- It should not produce any adverse effect.[13]

Types

There are two types of gene therapy:

- Somatic gene therapy – This procedure includes introduction of therapeutic genes into the somatic cells, which results in confinement of the effects, and hence, it is not carried onto the successive generation.[14]
- Germ line gene therapy – This procedure includes alteration of the sperm or the egg by the introduction of the therapeutic gene that gets integrated in the genome.[15]

Modes of delivery of therapeutic genes

There are two ways in which a therapeutic material can be transferred into the target cells. They are as follows:

1. *Ex vivo*: In this method, the therapeutic material can be implanted inside the cells of the diseased tissue outside the body, and then it is placed inside the body. This method is not much used in the treatment of oral cancer.
2. *In vivo*: In this method, the therapeutic material is directly implanted into the body at the diseased site. Here, the therapeutic materials are delivered into the target cells by a delivery vehicle, known as a vector.[13]

VECTORS AND ITS VARIANTS USED IN GENE THERAPY

Vectors can be defined as a vehicle, which is used to carry the therapeutic material into the diseased site. Vectors are of two types:

1. Viral Vectors: This comprises modified viruses, which can target and enter the diseased cell efficiently. Table 1 shows the modified viruses along with their advantages and disadvantages.[13]
2. Non-Viral Vectors: This can be broadly categorised into two parts, and they are as follows:

- Physical Methods: In these methods, the therapeutic gene is delivered into the target cell. This process is done by the following ways:

Table 1: Advantages and disadvantages of modified viruses

Virus	Advantage	Disadvantage
Retrovirus	Integration in genome ensures permanent expression Exogenous genetic material up to 9 kb is included High levels of expression It can be easily managed Transfer of gene can be done efficiently	Cells which are undergoing cell division are infected Transduction efficacy is low Titre is low There is a possibility of generating infectious viruses
Adenovirus	Cells which are undergoing cell division are infected Transduction efficacy is low Titre is high Insertion mutation is avoided as transmutation does not occur in the genome	Transitory expression Effectiveness of anti-adenoviral immunity is decreased, which requires episodic treatment Multiplication risk Lower expression levels Possibility of inflammatory and immune reactions
Adeno-Associated Virus	Transduction efficacy is high	Preparation is challenging
Herpes virus	Thymidine kinase expression High efficiency of gene transmission Large foreign DNA sequences can be inserted It can produce long-term dormant diseases Distribution of genes to pluripotent cells and their distinguished progeny are possible	Relatively toxic Gamma-herpes viruses are associated to malignity, occasionally

- Electroporation
- Gene gun
- Sonoporation
- Magnetifecation.

- Chemical Methods: In this method, a chemical delivery system is used to transfer the therapeutic gene. This process is done by the following ways:
  - Oligonucleotides
  - Lipoplexes and polyplexes
  - Dendrimers
  - Inorganic nanoparticles like gold, silica and iron oxides.[16]

TECHNIQUES EMPLOYED IN GENE THERAPY

Additional Gene Therapy: According to archival data, retinoblastoma gene; mutations of p53; p21; and p16 are used in genetic alteration of this procedure. In gene therapy, the mostly used tumour suppressor gene is p53 with an adenovirus as viral vectors as about 60% of tumour involves mutation of the p53 gene. In additional gene therapy, tumour suppressor genes are introduced, which help to inactivate the carcinogenic cells which gradually control the tumour growth.[17] According to the studies carried on Ad5CMV-p53, an adenovirus vector is first administered by intramucosal injection. After 2 h of the procedure, the use of mouthwash is advised. Following the succeeding day, it is administered for 2–5 days twice daily as mouthwash, which is repeated every 28 days. This technique not only has no toxic effects but also inhibits progression of disease in pre-malignant conditions. The literature shows that Rb gene and mda-7 are two other tumour suppressor genes that could be administered into the tumour for the same puprose.[18] According to the demonstrated data, gene p27 has the capability to trigger the suppression of the growth of tumour, prevent the cell cycle of tumour cells and induce apoptosis during gene transfer. Studies have shown that mutation of the p27 gene is related to tongue cancer. Hence, according to the studies, the use of p27 gene as the therapeutic gene might be proved useful for treatment of OSCC in future.[19]

Gene Excision Therapy: This therapy inhibits the growth of tumour cells by removing the defective oncogenes. One such example is the use of akadaic acid to suppress the carcinoma cells in OSCC. Highly toxic polyether i.e. akadaic acid, inhibits phosphorylation of type 1 and 2A protein, which reduces the expression of Egr-1 and ultimately results in inhibition of tumour activity. Egr-1 inhibition might signify a noble method of treatment, since genes controlling cell cycle progression and cell growth, including those that encode for tissue factors PDGF-A, PTEN and TGF-β1 are controlled by the expression of this protein. According to the studies, inhibition of protein kinase C decreases the expression of the gene by increasing sensitivity of the tumour to radiotherapy.[20]

Antisense RNA Therapy: Introduction of therapeutic gene which helps to prevent the expression of diseased gene is known as antisense therapy. RNA that is complimentary to the gene-expressing DNA inhibits the gene expression.[21] Oncogenic activity including ras, fos and myc can be prevented by antisense RNA; and viruses, such as HTLV-1 (human T-lymphotropic virus) and HPV (human papillomavirus), HSV-1 can also be inhibited as well. The only con of this technique is the difficulty in the administration of sufficient amount of antisense molecules to prevent the tumour growth; hence, powerful promoters are being developed.[22]

Immunotherapy: Immunotherapy involves two pathways: increasing either the immunogenic potential of tumour cells or the patient's immune response to tumour. Immune cells, such as natural killer cells, cytokines and T-lymphocytes, manifest an inadequate function in patients with OSCC. Gottesman MM *et al*., in their study showed that a substantial reduction in the tumour was found due to increased activation of natural killer cells and cytolytic T lymphocyte when combined with mIL -12 (murine interleukin -12) and mIL –2 (murine interleukin 2) gene.[6] The data gathered from archives reveal that the expression of pro-inflammatory cytokines, e.g., IL-6, IL-1α and IL-8, and of enzymes that breaks down the matrix metalloproteinase-9 (MMP-9) is also increased by NF-kB. NF-kB is found to be an active component in the metastasis and progression of various cancers, including OSCC; and hence, inhibition of NF-kB may be useful in the synergetic treatment of oral cancer therapy.[23] Many different studies have shown anti-tumour effect by transduction of IL-2 when used with the mutated fibroblast of an adenovirus and RGD peptide (Adv-F/RGD).[24] High anti-tumoural effect is shown by Adv-F/RGD due to its effect on the targeted cells or tissues, which include high necrotic changes and increased mononuclear cell infiltration, thus controlling the metastasis, or spread of the cancer cells. This is important since most of the deaths in cancer occur due to metastasis.[25]

Suicide Gene Therapy: Here, the therapeutic gene is introduced into the cell, which permits the expression of enzymes to transform non-toxic drugs into cytotoxic substances. It is the most used gene therapy, which makes use of thymidine kinase. The literature shows the

administration of HSVtk gene via an adenovirus vector combined with ganciclovir is beneficial for treatment of OSCC.[26] Deficient distribution of the vector inside the tumour is one of the main drawbacks of the gene therapy.[27]

Use of Oncolytic Genes: This can be the most recommended approach of gene therapy, in which the vector is genetically modified, that replicates the tumour cells. This therapy was developed from the detection of adenoviruses deficient of E1B, which did not grow in normal cells but grow in cells deprived of p53.[28] According to the studies, significant reduction of the tumour and regional metastasis is observed in primary tumours after its surgical excision due to the release of an oncolytic herpes virus.[29] Cirone P *et al*. in their studies reported intravenous injection of oncolytic adenovirus OAS403 have an anti-tumoural efficacy.[30]

Gene Therapy for Tumour Angiogenesis: In this technique, therapeutic proteins are released to encapsulate recombinant cells. These cells secrete angiostatin, which is an angiogenic inhibitor. ATP synthase is presented by angiostatin receptors superficially on the human endothelial cells such as in αvβ3-integrin and vitronectin. As a result, angiostatin remains localised inside the tumour rather than in organs close to the implantation of the capsule. When the tumour is in the advanced stage, this technique is not much of use. This therapy is time consuming as well as requires repetitive doses and is also related with an increased level of toxicity.[31] According to studies, the development of vaccines in contrary to receptor 2 of the VEGF (vascular endothelial growth factor), likely called as FLK 1, resulted in inhibition of tumour growth, metastasis and angiogenesis.[31] Kumar NA *et al*. in their study demonstrated that the vaccine against FLK 1 is effective, as it stimulates the T lymphocytes deactivating the receptor, which leads to the vascularisation of the tumour. Also, a treated case with this vaccine revealed an increase in the immune response at 10 months of inoculation of tongue metastasis of OSCC.[32]

New trends relating to the use of gene therapy in the case of OSCC

The new status in quo is the use of or intervention of nanoparticles in gene therapy. The general procedures employed in terms to OSCC include the following[33]:

- Up-regulation of genes inhibiting tumour growth or down-regulation of genes which promote tumour growth
- Suicide gene therapy
- Delivery of small RNA which targets the drug resistant genes.

The archives show that Xu M *et al*. in their studies revealed how they introduced or delivered Wnt-1 small RNA (siRNA), which ultimately inhibited the expression of epithelial-mesenchymal transformation (EMT) related gene into cytoplasm, which resulted in the restriction of invasion and migration of tumour cells.[33]

To increase the efficiency of suicide gene therapy, magnetic nanoparticles have been widely employed as a better vehicle. The archives reveals that in the year 2014, the use of PEI-modified Fe_3O_4 nano-particles to mediate transfection in OSCC by human-TRAIL gene driven with a human telomerase reverse transcriptase tumour-specific promoter (pACTERT-TRAIL) which induces apoptosis was performed to observe the antitumor function of the same in both *in vitro* and *in vivo*. They conferred that in comparison to the conventionally used PEI/lipofectin, the pACTERT-TRAIL nanoparticles showed a higher rate of transfection efficiency and mediated the killing of Tca-8113 cells.[33]

However, the major problem remained that of the multidrug resistance demonstrated by the cancer patients. To overcome this situation, the scientists and researchers across the globe came up with a procedure to prevent or reverse the effect of the multidrug resistance. The multidrug resistance protein 1 (MDR1) is a cell membrane, which is involved in pumping the foreign substance out of the cells. The data gathered from archives show that of higher level of MDR1 in OSCC patients is detected, which is the main cause of the drug resistance in such patients causing failure of on-going treatment of the patients. In a study, Li H *et al*. revealed how they fabricated mesoporous silica nanoparticles encapsulated with doxorubicin and MDR1-siRNA to block the MDR1 expression and then transfected it to the tumour cells *in vitro*. According to them, this procedure could downregulate the expression of MDR1 and induce apoptosis of the targeted tumour cell.[33] Gene therapy approaches in oral cancer and precancerous lesions [Table 2][34]

Advantages of gene therapy

- Gene therapy prevents against the noxious effects in the body, which can be instigated by different therapies.[34]
- Defective gene can be replaced by a functional or therapeutic gene.[34]
- It costs less than various other therapies.[34]

Table 2: Gene therapy approaches in precancerous and cancerous lesions

Gene	Vector Used	Mechanism of Action	Mode of Administration	Author
MnSoD	Addition gene therapy	Decrease in peroxide flow suppresses tumour malignity and helps in cell mitosis	———	Liu *et al*., 1997
HSVtk gene	Suicide gene therapy	Upsurges apoptosis	———	Fukui *et al*., 2001
Anti- ICAM- 2	Immunotherapy	Complete regression of oral cavity tumours	———	Pérez *et al*., 2002
Mutated P53	Adenovirus ONYX-015	Rise in replication of cells with altered p53 (OSCC) is seen by using adenovirus or ONYX015	Intravenous injection Alone, plus 5-fluoracil or plus IL-2	Nemunaitis *et al*., 2003
Mutated P53	Adenovirus ONYX-015	Lessening of leukoplakias	Mouthwash- Adevexin®	Nemunaitis *et al*., 2000
MDR1, DHFR, MRP1	Suicide gene therapy	Modifies the immune system, decreases tumour angiogenesis and increases apoptosis	———	Gottesman, 2003
Intra-tumoural injection of Adv- F/RGD	Immunotherapy	Anti-tumour effect is increased by controlling the disease locally	———	Dehari *et al*., 2003
Alteration of Rb protein	OAS403	Expression of gene E4 is controlled and reduction in in-vivo and in-vitro toxicity	Alone or plus Doxil® (chemotherapeutic)	Ryan, 2004
4-1BB Gene	Immunotherapy	Activation of T lymphocytes	——	Cheuck *et al*., 2004

GT: Gene Therapy, MDR1: Multidrug resistant protein 1, MnSoD: Manganese Superoxide Dismutase, DHFR: Dihydrofolate-reductase, MRP1: Multidrug related protein, tKHSV: Thymidine kinase gene of the Herpes Simplex Virus

- Way of leading life of the patient is enhanced for future.[34]

Disadvantages of gene therapy

- Patients may have to experience numerous rounds of the therapy.
- Possibility of reduction of the host's response and immune system is present, which might affect the effectiveness of the gene therapy.
- Different types of probable problems to the patient, such as immune and inflammatory responses and toxicity, can occur due to the viral vectors.
- Introduction of DNA into an incorrect place in the genome, such as into a tumour suppressor gene, induction of a tumour can take place.[34]

CONCLUSION

Discovery of gene therapy came as a boon in the treatment of oral cancer, as it aims only the cancerous cells. In this era, the research on gene therapy is rising. In near future, gene therapy can become a reliable treatment option of oral cancer and pre-cancerous lesions. Gene therapy as a treatment option offers high effectiveness and less toxicity as compared to other therapies; and it also helps in decreasing the high mortality rates, which are generally related with cancerous and pre-cancerous lesions.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Sudbø J, Reith A. The evolution of predictive oncology and molecular-based therapy for oral cancer prevention. Int J Cancer 2005;115:339-45.
2. Chaudhary N, Gupta DK, Verma RK, Choudhary SR. Squamous cell carcinoma of tongue in a 12- year-old child. Acta Otolaryngol Case Rep 2016;1:87-9.
3. Leão ML, Sant'Ana MS, Mesquita RA, Gomez RS, Santos Silva AR, Khurram SA, *et al*. Oral squamous cell carcinoma frequency in young patients from referral centers around the world. Head Neck Pathol 2022;16:755-62.
4. Llewellyn CD, Johnson NW, Warnakulasuriya KA. Risk factors for squamous cell carcinoma of the oral cavity in young people-A comprehensive literature review. Oral Oncol 2001;37:401-18.
5. Sunil PM, Joseph I, Varghese SS. Gene therapy in oral squamous cell carcinoma – A short review. Oral Maxillofac Pathol J 2011;2:142-7.
6. Gottesman MM. Cancer gene therapy: An awkward adolescence. Cancer Gene Ther 2003;10:501-8.
7. Sunil PM, Ramachandran CR, Jaisanghar N, Santhosh Kumar C. Dental surgeons as gene therapist. Int J Oral Maxillofac Pathol 2012;3:65-7.
8. Tatum EL. Molecular biology, nucleic acids and the future of medicine. Perspect Biol Med 1966;10:1932.
9. Wigler M, Silverstein S, Lee LS, Pellicer A, Cheng Y, Axel R. Transfer of purified herpes virus thymidine kinase gene to cultured mouse cells. Cell 1977;11:22332.
10. Anderson WF, Killos L, SandersHaigh L, Kretschmer PJ, Diacumakos EG. Replication and expression of thymidine kinase and human globin genes microinjected into mouse fibroblasts. Proc Nat Acad Sci USA 1980;77:5399403.
11. Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M, *et al*. T lymphocyte- directed gene therapy for ADA- SCID: Initial trial results after years. Science 1995;270:47580.
12. Blau HM, Springer ML. Molecular medicine gene therapy: A novel form of drug delivery. N Engl J Med 1995;333:1204-7.
13. Indu S, Ramesh V, Oza N, Prashad KV. Gene therapy: An overview. J Orofacial Sci 2013;5:837.
14. Kumar MS, Masthan KM, Babu NA, Dash KC. Gene therapy in oral cancer: A review. J Clin Diagn Res 2013;7:12613.
15. Smith AE. Viral vectors in gene therapy. Annu Rev Microbiol 1995;49:807-38.
16. Singh R, Singh S, Kumar T, Kumar A, Kumar A, Nazeer J. A novel

approach in the treatment of oral cancer-Gene therapy an update. World J Pharm Pharm Sci 2017;6:2839.
17. Saraswathi TR, Kavitha B, Vijayashree Priyadharsini J. Gene therapy for oral squamous cell carcinoma: An overview. Indian J Dent Res 2007;18:1203.
18. Supriatno, Harada K, Kawaguchi S, Onoue T, Yoshida H, Sato M. Characteristics of antitumor activity of mutant type p27Kip1 gene in an oral cancer cell line. Oral Oncol 2004;40:679-87.
19. Okamura H, Morimoto H, Fujita M, Nasu F, Sasakia E, Haneji T. Suppression of Egr-1 expression in human oral squamous carcinoma cells by okadaic acid. Oral Oncol 2002;38:779-84.
20. Jacob KC, Yashoda R, Puranik MP. Gene therapy in the treatment and prevention of oral cancer: An overview. Int J Adv Health Sci 2015;2:1420.
21. Bertrand JR, Pottier M, Vekris A, Opolon P, Maksimenko A, Malvy C. Comparison of antisense oligonucleotides and siRNAs in cell culture and *in vivo*. Biochem Biophys Res Commun 2002;296:1000-4.
22. Tamatani T, Azuma M, Ashida Y, Motegi K, Takashima R, Harada K, *et al*. Enhanced radiosensitization and chemosensitization in NF-kappaB-suppressed human oral cancer cells via the inhibition of gamma-irradiation- and 5-FU-induced production of IL-6 and IL-8. Int J Cancer 2004;108:912-21.
23. Shillitoe EJ. Papillomaviruses as targets for cancer gene therapy. Cancer Gene Ther 2006;13:445-50.
24. Dehari H, Ito Y, Nakamura T, Kobune M, Sasaki K, Yonekura N, *et al*. Enhanced antitumor effect of RGD fiber-modified adenovirus for gene therapy of oral cancer. Cancer Gene Ther 2003;10:75-85.
25. Neves SS, Sarmento-Ribeiro AB, Simões SP, Pedroso de Lima MC. Transfection of oral cancer cells mediated by transferrin-associated lipoplexes: Mechanisms of cell death induced by herpes simplex virus thymidine kinase/ganciclovir therapy. Biochim Biophys Acta 2006;1758:1703-12.
26. Xi S, Grandis JR. Gene therapy for the treatment of oral squamous cell carcinoma. J Dent Res 2003;82:11-6.
27. Chahlavi A, Rabkin S, Todo T, Sundaresan P, Martuza R. Effect of prior exposure to herpes simplex virus 1 on viral vector-mediated tumor therapy in immunocompetent mice. Gene Ther 1999;6:1751-8.
28. Galanis E, Okuno SH, Nascimento AG, Lewis BD, Lee RA, Oliveira AM, *et al*. Phase I-II trial of ONYX-015 in combination with MAP chemotherapy in patients with advanced sarcomas. Gene Ther 2005;12:437-45.
29. Hawkins LK, Lemoine NR, Kirn D. Oncolytic biotherapy: A novel therapeutic plafform. Lancet Oncol 2002;3:17-26.
30. Cirone P, Bourgeois JM, Chang PL. Antiangiogenic cancer therapy with microencapsulated cells. Hum Gene Ther 2003;14:1065-77.
31. Niethammer AG, Xiang R, Becker JC, Wodrich H, Pertl U, Karsten G, *et al*. A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. Nat Med 2002;8:1369-75.
32. Kumar NA, Agrawal A, Sreedevi R. Novel therapy for oral cancer – Gene therapy an update. Br J Med Med Res 2015;9:18.
33. Li H, Zhang Y, Xu M, Yang D. Current trends of targeted therapy for oral squamous cell carcinoma. J Cancer Res Clin Oncol 2022;148:2169-86.
34. Sharma R, Khajuria R, Sharma CL, Kapoor B, Goswami KC, Kohli K. Gene therapy: Current concepts. JK Sci 2004;6:626.